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        Morphological and Functional Correlates

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The primary objective of experiments carried out during the fourth year was to determine the behavioral and morphological impact of systemic **hyperthermia** following moderate spinal cord injury. In these experiments moderate hyperthermia (39.5-40.0°C) was initiated 30 minutes post-injury for a period of four hours. Two days post-injury we initiated the behavioral assessment of locomotor function and at the conclusion of the study a histological analysis of tissue was carried out. Overall, the results support the original hypothesis of this proposal that whole body hyperthermia is capable of producing detrimental effects on functional recovery following traumatic spinal cord injury. During the fourth year we also carried out a study evaluating the effects of long term (12 hours) **hypothermia** on neurologic outcome measures. This study is important in addressing the question of most effective duration of hypothermic treatment following injury. This study is presently ongoing and should be completed within the next four months. In summary the research carried out during the past four years has provided behavioral and morphological evidence that moderate systemic hypothermia is an effective intervention that produces significant benefits in animals undergoing traumatic spinal cord injury. We have determined that these effects are most likely to result if the treatment is initiated within the first thirty minutes post-injury as there appears to be little or no beneficial effect when the treatment is initiated two hours post-injury. Likewise this treatment as delivered in the studies carried out was most effective against functional and anatomical deficits produced by moderate versus severe injury.

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THERAPEUTIC HYPOTHERMIA FOLLOWING TRAUMATIC SPINAL INJURY

ROBERT P. YEZIERSKI, PH.D.

INTRODUCTION

The research carried out during the funding period focused on the varied effects of systemic hypothermia/hyperthermia alone or in combination with neuroprotective agents on neurological outcome following SCI. In these studies we evaluated the effects of treatments on morphological and behavioral outcome measures following traumatic spinal cord injury. The premiss that lowering CNS temperature protects against the detrimental effects of hypoxia and ischemia evolved in the 1950's. The protective influence of moderate and severe hypothermia was first demonstrated in experimental models of both spinal and cerebral ischemia (Pontius et al.,1954,1955; Marshall et al.,1956; Rosomoff, 1954,1959), and its beneficial effects were concluded to be secondary to the lowering of cerebral metabolic demands. Based on early studies involving brain injury, hypothermic techniques were modified to provide local cooling to the injured spinal cord. As a result of this treatment dramatic recovery of neurologic function compared to animals without this treatment was reported. Based on these findings it was hypothesized that mild changes in cord temperature could affect the extent of injury occurring after spinal cord trauma. The rationale for this hypothesis centered around the fact that: (1) local spinal cord cooling has been shown to be beneficial; (2) modest decreases in CNS temperature are effective in models of cerebral and spinal ischemia; and (3) modest decreases in brain temperature in models of CNS ischemia protect against processes which have been implicated in the pathophysiology of SCI, e.g. alterations in the blood-brain barrier, edema formation, production of leukotrienes, and release of neurotoxic substances such as glutamate and aspartate (Dempsey et al. 1987, Dietrich et al. 1990, Busto et al., 1989). Recent findings of the neuroprotective effects of modest hypothermia in brain ischemia may be applicable to SCI and offer a treatment protocol with few complications. Indeed, preliminary observations suggest that modest temperature changes, such as can be produced via systemic hypothermia, can affect the degree of tissue injury following spinal trauma (Martinez and Green, 1992). The importance of such findings is that systemic hypothermia provides a simple approach by which the cord can be "cooled" and thus obviates the need for acute surgical intervention. If effective, modest systemic hypothermia provides an additional therapeutic approach that could be applied to the clinical treatment of acute SCI. Based on the above discussion it can therefore be concluded that there is sufficient justification in both the scientific and clinical literature for additional studies related to better defining the optimal parameters for the hypothermic treatment of the injured spinal cord. The experiments proposed in the original proposal were aimed at addressing a number of interrelated hypotheses focusing on defining the optimal hypothermic parameters required to produce neuroprotection and behavioral recovery in the injured spinal cord. Due to the influence of temperature on a wide range of important homeostatic mechanisms necessary for maintaining the structural and functional integrity of spinal tissue, it was proposed that increases in systemic or site of injury temperatures *(hyperthermia)* will accelerate the injury process and compromise behavioral recovery, whereas reducing the temperature *(hypothermia)* will result in neuroprotection and enhanced behavioral recovery. We also proposed that there is an optimum time (post-injury) when post traumatic hypothermia of injured tissue produces the greatest benefit and is most effective in reducing behavioral deficits and morphological damage. Finally, there is also an optimum duration of hypothermic treatment which results in the greatest benefit to neurologic outcome. Experimental results related to these questions are described below.

RESEARCH ACCOMPLISHMENTS

STATEMENT OF WORK: FIRST YEAR

During the first year of funding one of the primary goals was to establish a therapeutic relationship between spinal cord temperature and neurologic outcome following traumatic spinal cord injury (SCI). In the studies carried out efforts were made to evaluate moderate hypothermia in order to determine if we could achieve neuroprotection following truamatic SCI. Although local spinal cord cooling had been attempted as a form of treatment in experimental and human SCI, most studies have focused on temperature shifts in the range of 15-20°C. Because of the technical difficulties required to achieve these conditions the application of hypothermia as a therapeutic intervention in SCI has been difficult to implement. Recent experimental data, however, suggests that modest changes (1-5°C) in central nervous system (CNS) temperature may positively influence outcome following CNS injury. The specific aim for the first year was, therefore, intended to utilize an established model of contusive spinal injury, i.e. weight drop, to evaluate the effects of hypothermic treatment on a morphological endpoint following injury. Since we would like to evaluate the neuroprotective effects of combination therapies, e.g. hypothermia with pharmacological treatment, we also initiated a study to evaluate the neuroprotective effects of neurotrophic factors and cytokines.

Experiment 1

Specific Aim: To determine the relationship between systemic and epidural temperatures in uninjured animals. Core (systemic) and spinal cord (epidural) temperatures were measured in an effort to establish the interdependence of these temperatures.

Rationale: In order to evaluate the therapeutic benefits of hypothermia in non-invasive treatment protocols it is important to determine the relationship between systemic and spinal cord temperatures.

Experimental Protocol: Rats were anesthetized, intubated, paralyzed and artificially ventilated. Heart rate and blood pressure were monitored in all animals throughout the length of the experiment and pO_2 and pCO_2 maintained within normal physiologic limits. Blood gases were evaluated every 30 minutes. Systemic temperature was measured by a teflon thermocouple probe inserted in the rectum. Epidural temperature was measured with a teflon thermocouple flexible probe. After a 30 minute control period epidural temperature was lowered by placing the animal in a plexiglas box with circulating thermal blanket. Systemic temperature was monitored continuously along with epidural temperature. The goal of this experiment was to determine the relationship between systemic and epidural temperatures within the range of 32-36°C (systemic temperature).

Results: During these experiments it was found that epidural temperature was slightly higher than rectal temperature. This was found to be the case when systemic temperature was at normothermic (36°C) levels as well as when systemic temperature was lowered to hypothermic levels (32°C). This relationship between epidural and systemic temperature is important as it provides information helpful to the design of studies where we will be evaluating specific target temperatures for cord cooling. Knowing the systemic temperature that one needs to obtain in order to achieve a specific epidural temperature will be necessary in order to evaluate the effects of different hypothermic conditions on morphological and functional outcomes.

Experiment 2

Specific Aim: The objective of this experiment was to study the effects of post-traumatic temperature manipulations on neurological outcome in animals subjected to traumatic SCI using mild and moderate contusive injury, and epidural temperatures of 32°C. Neurologic outcome was evaluated using morphological analysis of the injured cord.

Rationale: Using systemic cooling and the relationship determined in Experiment 1 the purpose of these experiments was to evaluate the question: 'will modest hypothermia reduce the severity of spinal cord injury resulting from contusive injury?' Our hypothesis is that modest lowering of cord temperature will lessen an animal's functional deficits and structural damage following injury.

Experimental Protocol: Rats were anesthetized, intubated, paralyzed, and artificially ventilated. Heart rate and blood pressure were monitored throughout the length of experiments and pO_2 and pCO_2 maintained within normal physiologic range. Epidural temperature was changed to maintain a temperature of 32°C (systemic temperature 30°C). Epidural temperature was monitored with a flexible thermocouple probe and contusive lesions produced by the weight drop technique (NYU impactor). Epidural temperature was lowered 30 minutes after injury and maintained at the same level for four hours. The time post-injury for application of hypothermic treatment along with the duration of treatment were both kept constant in this series of experiments. At the conclusion of experiments animals were deeply anesthesized with sodium pentobarbital and following fixation, spinal cord tissue was removed and prepared for histological examination, including determination of the volume of tissue damage.

Results: It was found that significant neuroprotection was achieved when the condition of moderate hypothermia was used following **moderate** spinal cord injury. No effects of hypothermia were observed under the conditions of these experiments following **severe** SCI. The results of this study showed that moderate hypothermia for 4 hours was capable of producing a decrease in contusion volume. The fact that there were no significant effects with severe injury indicates that for more severe injury it will be necessary to extend the period of hypothermia and/or increase the level of hypothermia (lower than 32°C). This latter condition is problematic since a hypothermic condition of this level will require a systemic temperature of less than 30°C which is a level that could compromise the survival of traumatically injured animals. It is for this reason that efforts will be made to extend the period of hypothermia (even if it means increasing slightly the epidural temperature to improve animal survival).

Experiment 3

Specific Aim: To identify the most appropriate time period (post injury) for the initiation of hypothermic treatment.

Rationale: It was hypothesized that there is an optimum time (post-injury) during which hypothermia is most effective in reducing morphological damage following contusive SCI. These experiments are important to establishing the "therapeutic window" during which benefits are derived from hypothermic treatment.

Experimental Protocol: Rats were anesthetized, intubated, paralyzed, and artificially ventilated. Heart rate and blood pressure were monitored in all animals throughout the length of the experiment and pO_2 and pCO_2 maintained within the normal physiologic range. Contusive injuries were produced by the weight drop technique. In these experiments animals were subjected to moderate injuries at a normal physiologic cord temperature

(37°C). Systemic temperature was changed to maintain the epidural temperature at a level of 32°C. This temperature was maintained for a period of four hours. The onset of the hypothermic condition was evaluated at 30 minutes and 2 hours post-injury. Spinal cord epidural temperature was monitored by a flexible teflon thermocouple probe. Following injury and treatment, animals were deeply anesthetized with sodium pentobarbital and transcardially perfused. Following fixation, spinal cord tissue was removed and prepared for histological examination and analysis including calculation of areas of gray and white matter damage.

Results: It was found that when body temperature was lowered commencing within 30 minutes post-injury significant neuroprotection was obtained. By contrast when hypothermia was initiated commencing 2 hours post-injury no effects of treatment were observed for a moderate level of SCI.

Experiment 4

Specific Aim: The goal of these experiments was to evaluate the neuroprotective effects of neurotrophic factors and cytokines following moderate contusion injury of the spinal cord.

Rationale: Based on the neuroprotective effects of neurotrophic factors and anti-inflammatory cytokines the desire to combine hypothermic and pharmacologic protocols to achieve neuroprotection, we evaluated the effects of intraspinal infusion of cytokines and neurotrophic factors on tissue damage following traumatic SCI.

Experimental Protocol: The neuroprotective effects of interleukins 1 (IL-1), 4 (IL-4), and 6 (IL-6), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor (bFGF) were evaluated in a contusion model of spinal cord injury. Female Sprague-Dawley rats (n=55) sustained a 10-gram weight drop injury to the lower thoracic spinal cord (T10) from a height of 12.5mm using the NYU impactor. A micro-infusion system (Alzet minipump) was used to continuously deliver drugs or saline directly into the epicenter of the contused spinal cord starting one or three hours post-injury. At the end of 7 days, animals were perfused for histopathlogical analysis. Longitudinal serial sections were cut on a freezing microtome and stained with cresyl violet. Areas of central necrosis, partial preservation, and total zone of tissue injury were traced by an independent reviewer using a computer based imaging system.

Results: In the vehicle-treated group (n=5), the mean volume of total injury (TZI) was $18.04 + 1.88mm^3$ and the mean volume of penumbral injury (ZPP) was $16.46 + 1.49mm^3$. Animals receiving interleukin-1 (IL-1) were found to have consistently larger TZI and ZPP volumes compared to control animals, though the difference was not significant (p>.05). It should be noted that despite similar postoperative care, the IL-1 treatment group also had a much lower survival rate (approximately 40%) than vehicle-treated and other treatment groups (approximately 90%). Interleukin 6 (IL-6) did not have any appreciable effects on any of the injury parameters. By contrast basic fibroblast growth factor (bFGF) was the most effective agent in reducing both the TZI (p=0.0004, ANOVA) and ZPP (p=0.0096, ANOVA). 2,3). Overall, bFGF reduced TZI by 33% and ZPP by 32% compared to control animals. Three other drugs were also found to have effects on contusion injury. Ciliary neurotrophic factor, NGF and IL-4 reduced TZI below the control group (ANOVA: CNTF, p=0.012; NGF, p=0.015; IL-4, p=0.016). CNTF infusion had the highest percentage reduction (23%) of these three drugs in terms of TZI (IL-4: 22%; NGF: 21%). Of these three factors, IL-4 reduced ZPP by 20% below control level, CNTF (17%). The reduction of ZPP by NGF was not statistically significant (p=0.083). In an effort to evaluate the potential therapeutic window of drug infusion, bFGF as the most effective treatment was selected for delivery three hours post injury. The comparison of this treatment with vehicle infusion showed no significant reduction in contusion volume.

CONCLUSIONS AND FUTURE DIRECTIONS

During the first year of funding we have established the relationship between systemic and epidural temperatures. Knowing this relationship will be important as we design experiments to further evaluate the optimum conditions of hypothermia to achieve neuroprotection and to test the effects of different hypothermic and hyperthermic conditions. We have also determined that there is a critical therapeutic window for the effects of moderate hypothermia delivered for a period of four hours post-injury. Since we also determined that moderate hypothermia delivered for four hours does not result in significant neuroprotection on severe injuries, it will be important to determine if this effect can also be improved with longer duration hypothermia. A critically important aspect of our work that will be addressed during the second year is to determine if there are functional correlates to the significant neuroprotection we have observed with hypothermia during the first year.

As one of the long term objectives of our research is the development of a total strategy of neuroprotection following SCI, we have begun to look at possible adjunct therapies that can be added to a hypothermic protocol. To this end, we determined that significant neuroprotective effects can be obtained with the intraspinal infusion of cytokines and neurotrophic factors commencing within one hour post-injury. No significant differences were observed between animals receiving saline versus bFGF treatment commencing 3 hours after injury. These data demonstrate that the continuous intramedullary infusion of bFGF, IL-4, NGF, or CNTF significantly reduces the total zone of injury, and for bFGF, IL-4, and CNTF the zone of partial preservation after moderate contusion injury of the spinal cord. These results support the further investigation and possible future clinical application of these agents as a solo treatment or in combination with hypothermia as a treatment strategy for acute spinal cord injury.

STATEMENT OF WORK: SECOND YEAR

During the second year of funding the primary goal was to establish a therapeutic relationship between spinal cord temperature and behavioral outcome measures following traumatic spinal cord injury (SCI). Studies carried out were intended to extend those carried out during the first year in which it was shown that moderate hypothermia delivered for a period of four hours was neuroprotective following moderate, but not severe, traumatic SCI. Although local spinal cord cooling had been attempted as a form of treatment in experimental and human SCI, most studies have focused on temperature shifts in the range of 15-20°C. Because of the technical difficulties required to achieve these conditions the application of hypothermia as a therapeutic intervention in SCI has been difficult to implement. Since we would like to ultimately evaluate the neuroprotective effects of combination therapies, e.g. hypothermia with pharmacological treatments, we also initiated a study to evaluate the neuroprotective effects of the endogenous neuroprotective agent agmatine, both alone and in combination with hypothermia. Agmatine has been shown to have neuroprotective properties in models of traumatic and ischemic head injury and excitotoxic spinal cord injury.

Experiment 1

Specific Aim: Evaluate the effects of mild hypothermia on locomotor function following traumatic spinal cord injury. The objective of these experiments was to study the effects of post traumatic hypothermia on behavioral outcome measures in animals subjected to traumatic SCI.

Rational: During the first year it was demonstrated that mild hypothermia delivered for a period of 4 hours thirty minutes after injury resulted in significant neuroprotection following traumatic SCI. An important question related to this effect is whether there is any behavioral significance attached to this neuroprotective effect.

Experimental Protocol: Rats were anesthetized, intubated, paralyzed and artificially ventilated. Heart rate and blood pressure were monitored in all animals throughout the length of the experiment and pO_2 and pCO_2 maintained within normal physiologic limits. Blood gases were evaluated every 30 minutes. Systemic temperature was measured by a teflon thermocouple probe inserted in the rectum. Epidural temperature was measured with a teflon thermocouple flexible probe inserted under the dura. After a 30 minute control period following injury the epidural temperature was lowered by placing the animal in a plexiglas box with circulating thermal blanket. Systemic temperature was monitored continuously along with epidural temperature throughout a 4 hour period during which the epidural temperature was maintained a level of either 37°C (normothremic) or 32°C (hypothermic). Two days following these procedures all animals were evaluated for residual locomotor function using the BBB locomotor rating scale. This evaluation continued using the schedule described above for the duration of the survival period (44 days). At the conclusion of experiments animals were deeply anesthetized with sodium pentobarbital and following fixation, spinal cord tissue was removed and prepared for histological examination, including determination of the volume of tissue damage.

Results: The results of this study showed that there were significant differences between the locomotor scores of animals in the hypothermic versus normothermic groups. There was a dramatic increase in BBB scores between days 2-12 post-injury for animals in both experimental groups. Starting on test day 19, however, hypothermic animals began showing significant differences in their BBB scores when compared to animals in the normothermic group. At the conclusion of the evaluation period it should be pointed out that the BBB scores for normothermic animals were beginning a trend in the downward direction while those for the hypothermic animals were trending in an upward direction. These results not only support the functional benefits of hypothermia, but also indicate that these beneficial effects are long term. In addition to evaluating locomotor scores during the survival period tissue was taken from all animals and subjected to a rigorous analysis of tissue damage. The results showed a significant reduction in the area of tissue damage in animal undergoing hypothermic treatment.

Experiment 2

Specific Aim: Evaluate the effects of the systemic administration of agmatine on locomotor function following traumatic spinal cord injury. The objective of this series of experiments was to study the effects of agmatine on morphological and behavioral outcome measures in animals undergoing traumatic SCI.

Rationale: Previously it has been shown that agmatine is neuroprotective in models of CNS injury (trauma and ischemia). Our work has shown that agmatine administered systemically or intraspinally also produces significant neuroprotective effects against excitotoxic tissue damage produced by intraspinal injections of quisqualic acid. Considering the fact that agmatine is an NMDA antagonist and an inhibitor of NOS, we wanted to determine if agmatine could produce neuroprotective and/or behavioral effects following traumatic SCI. Based on our long term goals of combining therapeutic strategies with hypothermia it was also thought that this evaluation was an important first step towards accomplishing this goal with a substance that could be systemically administered.

Experimental Protocol: Rats were anesthetized, intubated, paralyzed, and artificially ventilated. Heart rate and blood pressure were monitored throughout the length of experiments and pO_2 and pCO_2 maintained within normal physiologic range. Epidural temperature was maintained at the normothermic level of 37°C (systemic temperature 37°C). Epidural temperature was monitored with a flexible thermocouple probe and contusive lesions produced by the weight drop technique (NYU impactor). The time post-injury for the first injection of agmatine was 15 minutes. Animals received agmatine injections 100mg/kg every day for 14 days during the post injury survival period. At the conclusion of experiments animals were deeply anesthesized with sodium pentobarbital and following fixation, spinal cord tissue was removed and prepared for histological examination, including determination of the volume of tissue damage.

Results: The results of this study showed that agmatine produced significant differences in locomotor scores when compared to animals in the normothermic group. There was a significant improvement in BBB scores between days 2-9 post-injury for animals in the agmatine group. A comparable improvement in scores for animals in the normothremic group did not occur until day 16 post injury. Throughout the entire evaluation period the BBB scores for the agmatine animals were significantly higher for the agmatine group compared to animals in the normothermic group. In addition to evaluating locomotor scores during the survival period tissue was taken from all animals and subjected to volume analysis of tissue damage. The results of this analysis showed that animals treated with agmatine had significantly less damage than saline treated control animals.

Experiment 3

Specific Aim: Evaluate the effects of mild hypothermia plus the administration of agmatine on locomotor function following traumatic spinal cord injury. The objective of these experiments was to evaluate the effects of post traumatic hypothermia plus systemically administered agmatine on behavioral outcome measures in animals subjected to traumatic SCI and epidural temperatures of 32°C.

Rationale: Considering the positive effects of hypothermia or agmatine on behavioral outcome measures following traumatic SCI, the hypothesis was proposed that by combining these two interventions there may be a synergistic or additive effect of these two interventions.

Protocol: A protocol combining those described in Experiments 1 and 2 (above) was used in this study.

Results: The results of this combination therapy showed that there were significant effects at early and late time points for animals in the hypothermia + agmatine group compared to the BBB scores of animals in the normothermic group, but no evidence was observed of an additive or synergistic effect of these two interventions. This conclusion is based on the similarity of final BBB scores obtained in the hypothermia, agmatine and hypothermia + agmatine groups. Although the results of this study didn't show any advantage of combining hypothermia with agmatine one effect hypothermia may have is to extend the therapeutic window of agmatine. To test this hypothesis one would need to administer agmatine at different time points during the period of hypothermia and determine if similar results to agmatine administered at 30 minutes post injury would be obtained. This project will be considered for the future. The results of this series of experiments leads to the conclusion that agmatine, which blocks NMDA receptors and inhibits NOS, is only able to provide behavioral results equal to that observed with hypothermia. Furthermore, it is suggested that the effects of agmatine and hypothermia are working through a comparable mechanism. For there to be an additive effect of pharmacological treatment and hypothermia it is suggested that a drug affecting another component of the secondary injury cascade must be used. One possibility is IL-10 which has been shown to produce neuroprotection in traumatic SCI by affecting the inflammatory component of the injury cascade.

CONCLUSIONS AND FUTURE DIRECTIONS

During the second year of funding we determined that mild hypothermia (32°C) delivered within thirty minutes of a moderate injury for a period of four hours produces significant differences in locomotor scores when compared to normothermic animals. This result combines findings from the first year in which we determined the most effective duration of treatment, onset time, and injury severity required to produce neuroprotective effects of hypothermia. In a parallel series of experiments we determined that administration of the NMDA antagonist and NOS inhibitor agmatine also produces significant neuroprotective effects against excitotoxic injury resulting from the intraspinal injection of quisqualic acid. These effects were achieved with either intraspinal or systemic administration of agmatine. The encouraging results of this study led to a series of experiments closer to the hypothermia research plan and included the evaluation of agmatine effects against traumatic SCI. In these experiments we determined that the systemic administration of this drug produced significant behavioral effects. Because these effects could be achieved with systemic administration of the drug, unlike the effects of cytokines

and growth factors evaluated in the first year and because of our desire to combine hypothermia with a pharmacological intervention , the results with agmatine led us to the evaluation of combining hypothermia with the systemic administration of agmatine. Unfortunately, the combination of hypothermia and agmatine did not result in an additive or synergistic effect on behavioral outcome suggesting that hypothermia should be combined with an intervention directed at another component of the secondary injury cascade.

STATEMENT OF WORK: THIRD YEAR

During the third year the primary goals were to complete the evaluation of the effects of hyperthermia and hypothermia on behavioral and morphological outcome measures following traumatic spinal cord injury (SCI). Although progress had been made during year two, it was clear that we had significantly underestimated the time frame needed to complete these studies. Due to the labor intensive nature of carrying out the behavioral testing (for a period of 44 days post-injury), the need to histologically process and cut serial sections from each animal, and the time involved in doing volume analysis of these sections it was clear that additional time was needed to complete the studies initiated in year two. Additionally, we wanted to continue our evaluation of the effects of agmatine and agmatine plus hypothermia on these outcome measures. These studies were intended to extend those carried out during the second year in which it was shown that moderate hypothermia delivered for a period of four hours was neuroprotective following moderate, but not severe, traumatic SCI. The specific aims for year three were intended to utilize a clinically relevant model of contusive spinal injury, i.e. weight drop, and evaluate the effects of systemic hypothermic treatment on morphological and behavioral endpoints following injury.

Experiment 1

Specific Aim: Evaluate the effects of mild hypothermia on locomotor function following traumatic spinal cord injury. The objective of these experiments was to study the effects of post traumatic hypothermia on behavioral outcome measures in animals subjected to mild traumatic SCI.

Rational: During the first year it was demonstrated that mild hypothermia delivered for a period of 4 hours thirty minutes after injury resulted in significant neuroprotection following traumatic SCI. An important question related to this effect is whether there is any behavioral significance attached to this neuroprotective effect.

Experiment 2

Specific Aim: Evaluate the effects of the systemic administration of agmatine on locomotor function following traumatic spinal cord injury. The objective of this series of experiments was to study the effects of agmatine on morphological and behavioral outcome measures in animals undergoing traumatic SCI.

Rationale: Previously it has been shown that agmatine is neuroprotective in models of CNS injury (trauma and ischemia). Our own work has shown that agmatine administered systemically or intraspinally also produces significant neuroprotective effects against excitotoxic tissue damage produced by intraspinal injections of quisqualic acid. Considering the fact that agmatine is an NMDA antagonist and an inhibitor of NOS, we wanted to determine if agmatine could produce neuroprotective and/or behavioral effects following traumatic SCI. Based on our long term goals of combining therapeutic strategies with hypothermia it was also thought that this evaluation was an important first step towards accomplishing this goal with a substance that could be systemically administered..

Experiment 3

Specific Aim: Evaluate the effects of mild **hyperthermia** on locomotor function following traumatic spinal cord injury. The objective of these experiments was to evaluate the effects of post traumatic hyperthermia (39.5-40.0°C) on behavioral outcome measures in animals subjected to traumatic SCI.

Rationale: Considering the positive effects of hypothermia on behavioral outcome measures following traumatic SCI, the hypothesis was proposed that the opposite effect, i.e. increasing the temperature, would have a detrimental effect on behavioral and morphological outcome measures.

Protocol: A protocol combining those described for experiments carried out during years 1 and 2 was used in this study.

Results: The results have shown that mild hyperthermia results in significant deterioration of spinal tissue compared to control animals. In our analysis of differences in tissue damage in two groups of animals we have observed a 10-15% difference in the overall area of damage between these two groups of animals. Additionally, there were significant effects at early and late time points for animals in the hyperthermia group compared to the BBB scores of animals in the normothermic group. This conclusion is based on the significant difference in the final BBB scores obtained in the hyperthermia vs. normothermia.

CONCLUSIONS AND FUTURE DIRECTIONS

During the third year of funding we determined that mild hypothermia (32°C) delivered within thirty minutes of a moderate injury for a period of four hours produces significant differences in locomotor scores when compared to normothermic animals. This result combines findings from the first and second years in which we determined the most effective duration of treatment, onset time, and injury severity required to produce neuroprotective effects of hypothermia. In a parallel series of experiments not directly related to the research plan of the hypothermia project we also determined that administration of the NMDA antagonist and NOS inhibitor agmatine also produces significant neuroprotective effects against excitotoxic injury resulting from the intraspinal injection of quisqualic acid. These effects were achieved with either intraspinal or systemic administration of agmatine. The encouraging results of this study led to a series of experiments closer to the hypothermia research plan an included an evaluation of agmatine effects against traumatic SCI. In these experiments we determined that the systemic administration of this drug produced significant behavioral effects. Because these effects could be achieved with systemic administration of the drug, unlike the effects of cytokines and growth factors evaluated in the first year which were obtained with intraspinal injection and our desire to combine hypothermia with a pharmacological intervention , the results with agmatine led us to the evaluation of combining hypothermia with the systemic administration of agmatine. Unfortunately, the combination of hypothermia and agmatine did not result in an additive or synergistic effect on behavioral outcome suggesting that hypothermia should be combined with an intervention directed at another component of the secondary injury cascade.

STATEMENT OF WORK: FOURTH YEAR

In the fourth year of funding (no cost extension year) there were two objectives that we wanted to accomplish: (a) evaluate the effects of 12 hours of hypothermia treatment on neurological outcome; and (b) complete the evaluation of the effects of hyperthermia on behavioral and morphological outcome measures.

Experiment 1

Evaluate the effects of 12 hours of hypothermia commencing within 30 minutes of injury. To date our research has shown that mild hypothermia delivered for a period of 4 hours produces significant behavioral effects. To determine if a longer duration of hypothermic treatment is capable of producing an even greater morphological

and behavioral effect we believe it is possible to carry out a study using the 12 hour time frame. At the present time this study is still ongoing and all animals will be sacrificed by the end of January, 2001. We are carrying out BBB (behavioral; locomotor) testing to determine the effects of long duration hypothermia on a functional outcome measure. Once the animals are eliminated we will process the spinal cords to determine the extent of neuroprotection achieved relative to those in a previous study where animals under went 4 hours of hypothermic treatment. It is anticipated that the analysis of behavioral and morphological data from this study will be completed by May, 2001.

Experiment 2

One of the goals of the original research plan was to evaluate the effects of hyperthermia on morphological and behavioral outcome measures. To complete our evaluation of the effects of temperature on recovery from spinal cord injury, i.e. hypothermia and hyperthermia, we would like to complete our evaluation of the effects of hyperthemia on morphological and behavioral outcome measures..

During the fourth year we had, in addition to the above projects, wanted to initiate studies focusing on the physiological objectives of the original research plan. In the original proposal we had outlined experiments in which efforts would be made to determine the physiological correlate for any increased anatomical or behavioral improvement. Unfortunately, during this year we were forced to prematurely curtail our research activities while preparations were made to move the research laboratories of the The Miami Project to a new building. The Pope Life Center is the new home of The Miami Project and because of this move we were unable to initiate these studies. In addition to the disruption of moving my laboratory, the PI has also accepted a new position at the University of Florida. For this reason once the laboratory equipment was packed for the move to the new building it was then stored in preparation for the subsequent move to the University of Florida. Another consequence of these circumstances was the departure of two post-doctoral fellows which prevented the initiation of new research endeavors. Because of the inability to complete the proposed studies it is likely that there will be funds remaining at the end of the no-cost extension. Any unused funds will be returned to the funding agency.

KEY RESEARCH ACCOMPLISHMENTS AND CONCLUSIONS

● Four hours of mild systemic *hypothermic* treatment produces significant behavioral and morphological improvement versus animals receiving normothermic treatment following moderate and NOT severe traumatic spinal cord injury.

● Four hours of mild systemic *hyperthermic* treatment produces significant behavioral and morphological deficits compared to animals receiving normothermic or hypothermic treatment.

● Initiation of hypothermic treatment 30 minutes post-injury is significantly more beneficial than starting treatment two hours post-injury. Likewise hypothermic treatment is significantly more effective in the treatment of moderate versus severe injury.

● Animals receiving systemic injections of the NOS inhibitor and NMDA antagonist (agmatine) have significantly better morphological and behavioral outcome measures compared to vehicle treated control animals.

● There are no synergistic effects of agmatine + hypothermia following traumatic spinal cord injury.

● Infusion of selected growth factor and cytokines into the site of traumatic injury results in a marked improvement in morphological and locomotor outcome following traumatic spinal cord injury.

● There exists a direct relationship between epidural and rectal temperature so that it is possible by monitoring rectal temperature one has a good indication of epidural temperature.

REPORTABLE OUTCOMES

PAPERS:

1.Yu, CG, Marcillo, AE, Dietrich, WD, Fairbanks, CA, Wilcox, GL, Yezierski, RP **Agmatine improves locomotor function and reduces tissue damage following traumatic spinal cord injury in rats.** Neuroreport 11:3203-3207, 2000.

2. Yu, CG, Jimenez, O, Marcillo, AE, Weider, B., Bangerter, K, Dietrich, WD, Castro, S, Yezierski, RP **Beneficial effects of modest systemic hypothermia on locomotor outcome and histopathological damage following contusion spinal cord injury in rats.** J. Neurosurg. 93:85-93, 2000.

3. Lee, TT, Dietrich, WD, Green, BA, Yezierski, RP **Neuroprotective effects of basic fibroblast growth factor following spinal cord contusion injury in the rat.** J. Neurotrauma 16:347-356, 1999.

4. Yu, CG, Jimenez, O, Marcillo, AE, Weider, B., Bangerter, K, Dietrich, WD, Castro, S, Yezierski, RP **Detrimental effects of modest systemic hyperthermia on locomotor outcome and histolpathological damage following contusion spinal cord injury in rats.** Neurosurgery (submitted).

5. Yu, CG, Dietrich, WE, Marcillo, AE, Yezierski, RP **Beneficial effects of 12 hours of moderate hypothermic treatment following traumatic spinal cord injury: morphological and functional analysis.** J. Neurosurgery (in preparation).

ABSTRACTS:

1. Yu, CG, Marcillo, AE, Sanchez,D, Ruenes, GR, Jagid, J, Dietrich, WD, Yezierski, RP **The effects of hyperthermia on locomotor outcome and histopathology following traumatic spinal cord injury in the rat.** J. Neurotrauma, 17:464, 2000.

2. Yu, CG, Marcillo, AE, Dietrich, WD, Fairbanks, CA, Wilcox, GL, Yezierski, RP **Beneficial effects of hypothermia and/or agmatine on locomotor outcome following contusion spinal cord injury.** Neurosci. Abstr., 25:1443, 1999.

3. Jimenez, O, Weider, BN, Bangerter, K, Dietrich, WD, Martinez-Arizala, A, Yezierski, RP. **Effects of systemic hypothermia following acute traumatic spinal cord injury in the rat.** J. Neurotrauma 14:772, 1997.

CONCLUSIONS

It is suggested from the work carried out during the past four years that field administration of hypothermic treatment immediately after traumatic injury to the spinal cord can potentially lead to the expansion of the therapeutic window for the delivery of pharmacological interventions and/or to an improved neurological outcome (behavioral and morphological). Development of field devices that can lower systemic temperature following injury (head or spinal cord) is recommended and a full scale clinical trial is suggested by the results obtained in the research obtained during the past four years. It is also recommended that immediate steps be taken to combat elevations in systemic temperature due to fever, infection, etc. following injury as even slight elevations can lead to a significant detrimental affect on neurological outcome. The results also determined that future therapeutic agents with multiple pharmacologic targets like agmatine (an NMDA antagonist and NOS inhibitor) are potentially more beneficial than drugs with singular pharmacologic effects. Finally, spinal infusion of growth factors and protective cytokine is potentially an adjunct therapeutic intervention for acute spinal cord injury. Additional studies are indicated to evaluate the effects of hypothermia on the therapeutic window as well as related to the interaction of hypothermic treatment and IL-10, methylprednisilone and perhaps other pharmacologic agents directed at different components of the post-injury secondary cascade.

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APPENDICES

PAPERS:

1.Yu, CG, Marcillo, AE, Dietrich, WD, Fairbanks, CA, Wilcox, GL, Yezierski, RP **Agmatine improves locomotor function and reduces tissue damage follwoing traumatic spinal cord injury in rats.** Neuroreport 11:3203-3207, 2000.

2. Yu, CG, Jimenez, O, Marcillo, AE, Weider, B., Bangerter, K, Dietrich, WD, Castro, S, Yezierski, RP **Beneficial effects of modest systemic hypothermia on locomotor outcome and histopathological damage following contusion spinal cord injury in rats.** J. Neurosurg. 93:85-93, 2000.

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Agmatine improves locomotor function and reduces tissue damage following spinal cord injury

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Clinically effective drug treatments for spinal cord injury (SCI) remain unavailable. Agmatine, an NMDA receptor antagonist and inhibitor of nitric oxide synthase (NOS), is an endogenous neuromodulator found in the brain and spinal cord. Evidence is presented that agmatine significantly improves locomotor function and reduces tissue damage following traumatic SCI in rats. The results suggest the importance of future therapeutic strategies encompassing the use of single drugs with multiple targets for the treatment of acute SCI. The therapeutic targets of agmatine (NMDA receptor and NOS) have been shown to be critically linked to the pathophysiological sequelae of CNS injury and this, combined with the non-toxic profile, lends support to agmatine being considered as a potential candidate for future clinical applications. *NeuroReport* 11:3203–3207 © 2000 Lippincott Williams & Wilkins.

Key words: Agmatine; Locomotor function; Neuroprotection; Nitric oxide; NMDA

INTRODUCTION

The pathological sequelae associated with traumatic SCI include white and gray matter damage resulting from the primary injury and a progressive secondary injury cascade that begins with the elevation of excitatory amino acids [1]. The NMDA receptor (NMDAR) and nitric oxide synthase (NOS) are known to play important roles in the progression of secondary injury initiated by brain and spinal cord injury [2–4]. Agmatine (decarboxylated arginine) is an endogenous neuromodulator found in brain [5] and spinal cord [6] with both NMDAR antagonist [6,7] and NOS inhibitor activities [8,9]. Pretreatment and/or treatment with agmatine prevents the development of opioid tolerance [10], reduces infarct size after global cerebral ischemia [11], attenuates the extent of neuronal loss following excitotoxic spinal cord injury [6], and alleviates pain behaviors associated with excitotoxic lesions in the spinal cord [6]. Based on this evidence, we hypothesized that agmatine may have neuroprotective effects following traumatic SCI. The purpose of the present study was to evaluate the effects of agmatine on locomotor function and histopathological damage following traumatic injury using a clinically relevant model of SCI.

MATERIALS AND METHODS

Experimental procedures were approved and carried out in accordance with the Guidelines of the Animal Care and Use Committee of the University of Miami.

Surgical preparation: Under halothane anesthesia (4% in 70% N_2O, balanced with O_2), 23 female Sprague–Dawley rats weighing 225–275 g had their neck and back shaved and scrubbed with Betadine. Anesthesia was continued with halothane (0.5% for trauma, and maintenance). The antibiotic Crystiben (0.01 ml/100 g, i.m.) was administered to prevent iatragenic infection. A flexible thermistor was inserted in the rectum to monitor systemic temperature. Pre-SCI, all animals were maintained at a systemic temperature of 37°C (rectal) using a feedback controlled heating blanket. After injury animals were housed in cages containing soft bedding and placed on a thermal blanket (37.0°C) until thermoregulation was re-established. Animals were treated with Crystiben i.m. every other day for 7 days. Water bottles with extended tubes allowed access to water. Food was placed inside the cage until rats were capable of reaching the standard placement in the cage top. Animals were checked daily and bladders palpated at least twice daily and emptied as required until they regained reflex voiding.

Contusion spinal cord injury: Traumatic spinal injury was produced following a T10 laminectomy. Animals were

positioned in the weight-drop apparatus (NYU Impactor). Two spinal clamps were attached to T8/T9 and T11/T12 spinal processes, respectively. A transducer was placed at the site of the muscle near the spinal column, and the impactor rod (10 g) centered above spinal segment T10. The rod was slowly lowered until it contacted the dura, which was determined by completion of a circuit that resulted in an audible tone. The cord was then contused with the NYU weight-drop device that released a 10 g rod from a height of 12.5 mm (moderate injury) onto the exposed cord. Impact analysis, including degree of cord compression, velocity, time, and height of weight drop, were recorded by a preset NYU impactor software package. After trauma, rats were randomized to agmatine and saline treatment groups.

Drug preparation and administration: Agmatine was purchased from Sigma (St. Louis, MO), dissolved in 0.9% saline and administered (100 mg/kg/day, i.p., 1 ml) 30 min post-trauma and once daily for 14 days. This dose was based on results of a previous dose–response determination using an excitotoxic model of SCI [6]. Higher doses (200 mg/kg, i.p., $n = 2$) may have shown toxic effects (e.g. blanching, shivering), but these effects were not studied systematically (Yezierski, unpublished observations).

Behavioral assessment: Open-field locomotor function was evaluated using the Basso–Beattie–Breshnahan (BBB) locomotor rating scale, a multiple function test of locomotor outcome that provides an efficient and unambiguous locomotor rating [12]. Briefly, non-injured rats were exposed daily for one week to the behavioral testing environment in order to acclimate them to open field exploration. Two examiners participated in the BBB evaluation and were positioned across from each other to observe both sides of the rat. Each rat was tested for 4 min. Postoperative open field testing for all animals occurred twice a week from day 2 post-injury to day 44 post-injury. Examiners were blinded to the type of treatment received by each animal.

Histopathological assessment: The method used to evaluate morphological outcome of spinal cords following injury was similar to that described in a recent study [13]. Briefly, 44 days post-SCI, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with isotonic saline for 5 min, followed by a mixture of 4% formaldehyde, glacial acetic acid and methanol (FAM) 1:1:8 by volume for 30 min. Following perfusion, the vertebral column with the cord were immersed in FAM at 4°C for 24 h. Spinal cords were then removed and embedded in paraffin in 16–18 mm long blocks that contained the contusion epicenter as well as non-injured tissue at both ends of each block. Each block was serially sectioned (longitudinally) at 10 μm. Sections were stained with hematoxylin, eosin and luxol fast blue for histopathological analysis. Sixteen sections from the central core of the cord were studied with light microscopy, and reconstructions of the longitudinal area of tissue damage in these sections were made with the aid of an overhead projector. The area of tissue damage in each section was quantitatively determined using computer-aided image analysis (Meta Morph

Imaging System, Universal Imaging Corporation). The sum of areas in the 16 sections analyzed was used as the total area of tissue damage [13].

Statistical analysis: All data are expressed as means ± s.e.m. and were analyzed using a commercially available computer program (StatView). For each rat, BBB scores for each hindlimb were averaged together to yield one score per test session. BBB scores and total area of damage were compared between the agmatine and saline groups at each time point using one-way analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) test. Differences were considered statistically significant at $p < 0.05$.

RESULTS
Injury parameters of the weight-drop device used to produce contusion injury are shown in Table 1. No significant differences in velocity, compression, height, and time were found between agmatine- and vehicle-treated groups, indicating similar injuries to all animals.

Behavioral assessment: Results of the BBB locomotor assessment are shown in Fig. 1. Immediately after SCI, all animals showed bilateral hindlimb paralysis, as previously documented using the NYU impactor [12,13]. Saline-trea-

Table 1. Contusion parameters ($n = 23$)

	Control	Agmatine-treated	p^a
Compression	1.71 ± 0.07	1.85 ± 0.03	ns
Height (mm)	12.48 ± 1.10	12.62 ± 0.08	ns
Velocity (m/s)	0.490 ± 0.002	0.489 ± 0.002	ns
Time (ms)	49.7 ± 1.54	50.8 ± 1.29	ns

Values are means ± s.e.m.
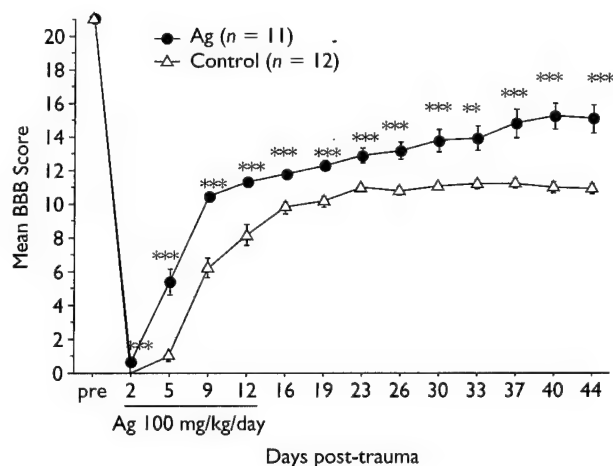^{a}ANOVA and Fisher's PLSD test.

Fig. 1. Time course of open-field locomotor recovery as measured by BBB locomotor scores following agmatine or saline treatment. Agmatine-treated animals are represented by the filled circles and saline-treated animals are represented by triangles. The solid bar indicates the time period of daily agmatine administration. Data are represented as mean ± s.e.m. $^{**}p < 0.01$; $^{***}p < 0.001$.

ted animals had little or no hindlimb movements 5 days post-trauma and then showed a gradual recovery over the next 2 weeks. By 3 weeks post-trauma, most saline-treated animals were stepping consistently but lacked forelimb–hindlimb coordination. There was little or no behavioral improvement in saline-treated animals between weeks 3 and 6 post-trauma. As early as 2 days post-trauma, agmatine-treated rats showed significantly more ($p < 0.001$) movement of their hindlimbs than did control animals. This difference continued throughout the duration of the survival period. Consequently, locomotor improvement observed in agmatine-treated animals was consistently greater than that observed in saline-treated animals at each time point evaluated (Fig. 1; Table 2). On day 44 after injury, the final mean BBB score for agmatine-treated animals was significantly higher than that of control animals (15.0 ± 0.83 vs 10.8 ± 0.29, $p < 0.001$).

Histopathological assessment: The results of the histopathological outcome assessment are shown in Fig. 2 and Fig. 3. Hematoxylin-, eosin- and luxol fast blue-stained sections showed that there was significant tissue loss at 44 days after traumatic SCI in the control group. By contrast the total area of tissue damage in agmatine-treated animals was significantly less than that of saline-treated animals.

Table 2. Mean BBB scores

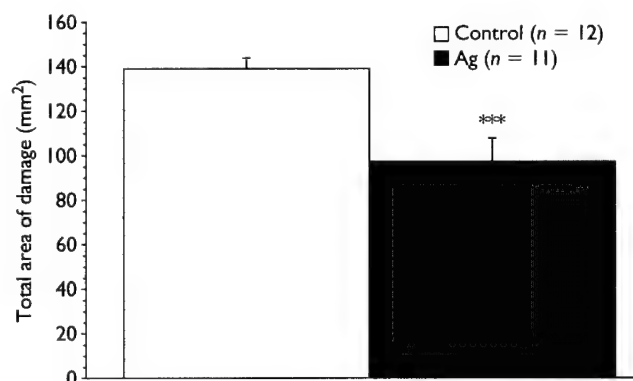| Days post-trauma	Agmatine ($n = 11$)	Control ($n = 12$)
2	0.68 ± 0.14	0.00 ± 0.00
5	5.36 ± 0.78	1.00 ± 0.24
9	10.45 ± 0.28	6.21 ± 0.60
12	11.27 ± 0.14	8.13 ± 0.61
16	11.73 ± 0.27	9.75 ± 0.31
19	12.27 ± 0.30	10.13 ± 0.33
23	12.82 ± 0.44	10.92 ± 0.20
26	13.09 ± 0.51	10.75 ± 0.18
30	13.73 ± 0.66	11.00 ± 0.20
33	13.86 ± 0.71	11.13 ± 0.26
37	14.73 ± 0.84	11.17 ± 0.27
40	15.14 ± 0.79	10.96 ± 0.35
44	15.00 ± 0.83	10.83 ± 0.29

Fig. 2. Area of spinal cord damage following agmatine or vehicle treatment. The area of tissue damage in each section was quantitatively determined using computer aided image analysis. Data are represented as mean $\pm$ s.e.m. *** $p < 0.001$.
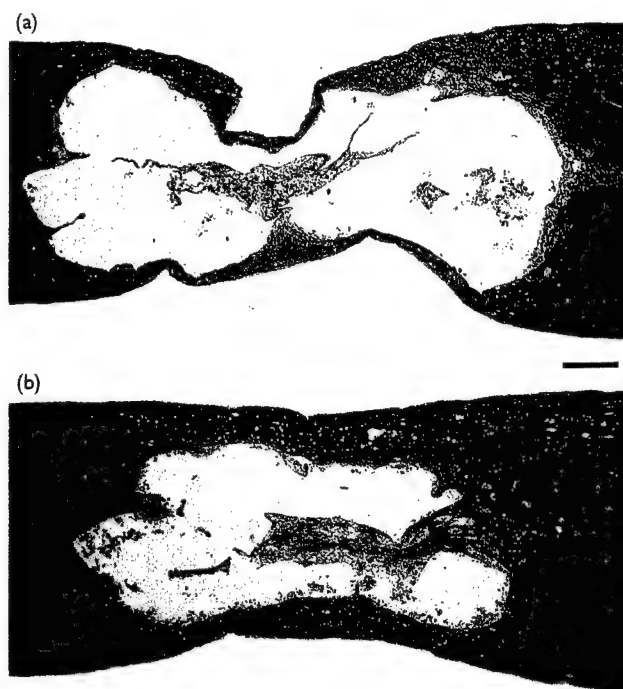

Fig. 3. Photomicrographs of representative histological sections following traumatic spinal cord injury and systemic treatment with either saline (a) or agmatine (100 mg/kg/day) (b). Note reduced area of tissue damage (b) following agmatine treatment. The area of injury was easily identified by the presence of reactive astrocytes, microglial, infiltration of inflammatory cell types (neutrophils, leucocytes) and by the presence of macrophages. Bar = 400 µm.

The final mean area of tissue damage in agmatine-treated animals was 30% less than that found in saline-treated animals ($p < 0.001$, Fig. 2). Sections representing the mean tissue damage from saline- vs agmatine-treated animals are shown in Fig. 3a,b, respectively. Note the reduced size of the cavity and partial sparing of white matter in the agmatine-treated section compared to the section from the saline-treated animal.

DISCUSSION

Traumatic SCI has been estimated to occur in 3/100 000 people, resulting in ~15 000 new cases/year [14]. At present the recommended treatment for acute SCI is the glucocorticosteriod methylprednisolone (MP) that is believed to have multiple actions including stabilization of membranes, reduction of edema, and an inhibitory effect on lipid peroxidation and free-radical reactions [15]. Unfortunately, many patients with spinal injury remain physically impaired as a result of their injury [14], and there is concern as to the therapeutic efficacy of MP [16]. For these reasons continued efforts are needed to develop and test novel treatment strategies directed at specific components of the post-injury cascade associated with SCI.

Experimental strategies designed to limit the extent of tissue damage following SCI have relied, in part, on the targeting of NMDA and non-NMDA receptors [2,17–19], the synthetic pathway for nitric oxide [20], or inflammation [21]. Injury-induced elevations in excitatory amino acids,

including glutamate, and the induction of NOS by NMDA receptor activation have been implicated as important steps in secondary injury following brain and spinal cord injury [1,22,23]. NMDA antagonist MK-801 (19) or the non-NMDA antagonist NBQX [17,18] is effective in reducing the extent of tissue damage and improving behavioral outcome following SCI. Although single drug pharmacotherapy is an important strategy for the treatment of acute SCI, the complex and dynamic processes initiated by SCI are characterized by the simultaneous occurrence and interactive nature of multiple pathophysiological events. The effective treatment of acute SCI may therefore require combination therapy with multiple drugs administered at different time points. An alternative to this strategy is the use of a single drug with multiple therapeutic targets. The present study supports this latter strategy by showing that agmatine, a NMDA receptor antagonist [7] and inhibitor of NOS [9], produces significant restoration of locomotor function and reduction of tissue damage following traumatic SCI in the rat.

In the present study, we demonstrated that agmatine (100 mg/kg/day, i.p., for 14 days) given 30 min after SCI significantly improves hindlimb motor function for up to 6 weeks following traumatic SCI. The most significant behavioral differences were consistent forelimb–hindlimb coordinated movements observed in agmatine-treated rats from 4 to 6 weeks post-injury, whereas control animals achieved only modest improvements without coordinated hindlimb movements. Our results also showed that exogenous agmatine significantly reduced the loss of spinal cord tissue 6 weeks following traumatic SCI as animals treated with agmatine had significantly greater tissue sparing than vehicle-treated animals. These data suggest that agmatine has strong neuroprotective effects on locomotor outcome and histopathological damage following traumatic SCI, effects that persisted for up to 30 days following cessation of treatment.

Previous studies have shown improvement of neurological outcome following the use of NMDA or non-NMDA antagonists. In the studies by Wrathall and colleagues acute administration of the non-NMDA antagonist NBQX, an AMPA/kainate receptor antagonist, produced an increase in speed and extent of recovery of hindlimb reflexes, coordinated motor function in swimming, overall locomotor function, bladder reflex, and enhanced positioning on an inclined plane [17]. Similar results were obtained with the delayed delivery of the drug [18]. Using the noncompetitive NMDA antagonist MK-801 significant improvement was observed in locomotor function, toe spread, and inclined plane scores [19]. Using multiple doses of gacyclidine, a specific phencyclidine analog and noncompetitive NMDA antagonist, Gaviria et al. [2] found a dose- and time-dependent attenuation of spinal cord damage after vascular lesions of the spinal cord. Motor recovery was related to the extent of preservation of spinal tissue at the level of injury. From these studies it is clear that both NMDA and non-NMDA mechanisms play a significant role in secondary injury and in producing subsequent behavioral deficits following SCI.

The mechanisms responsible for the neuroprotective effects of agmatine are believed to involve the selective blockade of the NMDA receptor [6,7] together with NOS blockade [8,9], both of which play an important role in secondary injury after brain and spinal cord injury [3,4,20,22,23]. This confluence of therapeutic mechanisms led us to predict that agmatine might have neuroprotective effects and thus improve locomotor outcome following SCI. The results of the present study support this hypothesis, but at the same time raise the question as to the additive or synergistic effects of NMDA receptor antagonists and inhibitors of NOS in SCI. Additional studies are needed to further address the importance of these two therapeutic targets in the pathological and functional consequences of SCI.

Consistent with the results of the present study Olmos et al. [24] reported agmatine is neuroprotective against glutamate-induced neurotoxicity. Treatment with agmatine also prevents the development of opioid tolerance [10], reduces infarct size after global cerebral ischemia [11], alleviates inflammatory and neuropathic pain behaviors associated with excitotoxic lesions in the spinal cord, and attenuates the extent of neuronal loss following quisqualate-induced spinal cord injury [6]. Since clinically effective drug treatments for acute SCI are still needed, new agents that prevent neurological damage and improve functional recovery need to be developed. The present study suggests a therapeutic strategy for the treatment of acute spinal cord injury: a single drug with multiple targets critically linked to the pathophysiological sequelae of spinal injury. The absence of acute motor toxicity after intrathecal (i.t.) administration of agmatine in mice suggests the existence of a large therapeutic index by the i.t. route of administration [6]. Agmatine may, therefore, represent a useful target for therapeutic development. As more is learned about the temporal profile of cellular and biochemical events responsible for the destruction of CNS tissue multi-action drugs (like agmatine) will need to be developed targeting different components of the secondary injury cascade.

CONCLUSION

The results of this study have shown that exogenous agmatine administered systemically for 14 days after spinal cord injury significantly improves locomotor function and reduces tissue damage. This action is consistent with a previous study showing that a single injection of agmatine following spinal nerve ligation reversed hypersensitivity indicative of pain [6]. Other studies have reported that agmatine prevents the development of opioid tolerance [10], reduces inflammation-induced thermal hyperalgesia [25] and reduces infarct size after global cerebral ischemia [11]. Furthermore, previous studies have suggested [11] or provided evidence [6] that agmatine's toxicity profile is very acceptable in rodents. The present results extend our observations regarding the neuroprotective effects of agmatine in excitotoxic lesions by demonstrating neuroprotection in a clinically relevant model of SCI as well as significant improvement in motor function. Importantly, there was continuous improvement in locomotor function for up to 30 days following cessation of agmatine treatment. Collectively, this long-term efficacy and low toxicity suggests that agmatine may be a promising therapeutic target for treatment of acute spinal injury. Finally, the cumulative evidence suggests that further investigation of agmatine with respect to therapeutic index, synergy of
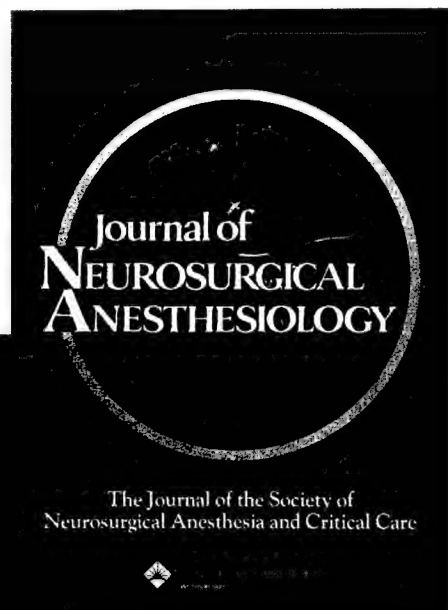
effects, and comparison with other currently used pharmacologic agents, e.g. MP, NBQX, interleukin-10, is warranted.

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Beneficial effects of modest systemic hypothermia on locomotor function and histopathological damage following contusion-induced spinal cord injury in rats

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Object. Local spinal cord cooling (LSCC) is associated with beneficial effects when applied following ischemic or traumatic spinal cord injury (SCI). However, the clinical application of LSCC is associated with many technical difficulties such as the requirement of special cooling devices, emergency surgery, and complicated postoperative management. If hypothermia is to be considered for future application in the treatment of SCI, alternative approaches must be developed. The objectives of the present study were to evaluate 1) the relationship between systemic and epidural temperature after SCI; 2) the effects of modest systemic hypothermia on histopathological damage at 7 and 44 days post-SCI; and 3) the effects of modest systemic hypothermia on locomotor outcome at 44 days post-SCI.

Methods. A spinal cord contusion (12.5 mm at T-10) was produced in adult rats that had been randomly divided into two groups. Group 1 rats (seven in Experiment 1; 12 in Experiment 2) received hypothermic treatment (epidural temperature 32–33°C) 30 minutes postinjury for 4 hours; Group 2 rats (nine in Experiment 1; eight in Experiment 2) received normothermic treatment (epidural temperature 37°C) 30 minutes postinjury for 4 hours. Blood pressure, blood gas levels, and temperatures (epidural and rectal) were monitored throughout the 4-hour treatment period. Twice weekly assessment of locomotor function was performed over a 6-week survival period by using the Basso-Beattie-Bresnahan locomotor rating scale. Seven (Experiment 1) and 44 (Experiment 2) days after injury, animals were killed, perfused, and their spinal cords were serially sectioned. The area of tissue damage was quantitatively analyzed from 16 longitudinal sections selected from the central core of the spinal cord.

Conclusions. The results showed that 1) modest changes in the epidural temperature of the spinal cord can be produced using systemic hypothermia; 2) modest systemic hypothermia (32–33°C) significantly protects against locomotor deficits following traumatic SCI; and 3) modest systemic hypothermia (32–33°C) reduces the area of tissue damage at both 7 and 44 days postinjury. Although additional research is needed to study the therapeutic window and long-term benefits of systemic hypothermia, these data support the possible use of modest systemic hypothermia in the treatment of acute SCI.

KEY WORDS • spinal cord injury • spinal cord • locomotion • hypothermia • neuroprotection

LOCAL spinal cord cooling was first successfully applied in experimental SCI by Albin and colleagues.[1] Since this initial study the protective effects of local hypothermia have been demonstrated in different experimental models of SCI.[8,22,23,31,48] Clinical studies of LSCC are difficult to evaluate because they include only a few patients and lack randomized control groups.[37,50] In most experimental and clinical studies, the significant lowering (by 10°C) of spinal cord temperature presents many technical difficulties.

If local cooling of the spinal cord is to be used as a treatment for acute SCI, it will require special cooling devices, emergency surgery, and complicated postoperative management.[30,50] On the other hand, modest systemic hypothermia (30–33°C) has been shown to be an effective alternative to LSCC, having beneficial effects on functional and morphological outcome measures following ischemic and traumatic brain injury.[14,17,19–21,32,41] It can be concluded from these studies that the profound lowering of CNS temperature is not necessary for neuroprotection of tissue that is vulnerable to damage caused by the effects of traumatic or ischemic injury. Systemic hypothermia, even

moderate, prevents energy failure,[6] reduces histopatholog-ical damage,[20,40] and diminishes free radical activity and extracellular levels of glutamate following brain injury.[25] The application of systemic hypothermia also lessens neurological deterioration resulting from brain trauma,[11] improves neurological outcome following ischemic injury to the rat spinal cord,[36,45] and reduces polymorphonuclear leukocyte accumulation following traumatic SCI.[16]

Clinically, systemic hypothermia has been used to pre-vent ischemic injuries during open heart surgery[10] and as a treatment modality following ischemic and traumatic injury to the brain.[24,29] Moreover, the application of mild to modest hypothermia has been demonstrated to be benefi-cial in preventing tissue damage following traumatic SCI.[37] The importance of such findings is that, compared with LSCC, modest systemic hypothermia provides a much simpler approach by which the spinal cord can be cooled, thus reducing the need for acute surgical intervention. If effective, then modest systemic hypothermia could be used to provide an immediate, on-site, noninvasive therapeutic approach for acute SCI. The purpose of the present study was to investigate, under controlled physiological condi-tions, the influence of modest systemic hypothermia on locomotor function and histopathological damage by using a clinically relevant model of SCI.[3] A preliminary descrip-tion of this study has already been reported.[52]

Materials and Methods

Experimental procedures were approved and conducted in accor-dance with the Guidelines of the Animal Care and Use Committee of the University of Miami.

Experimental Design

The experimental design included an evaluation of the acute and chronic effects of systemic hypothermia on morphological and be-havioral outcome measures following traumatic SCI, which was conducted in two separate series of experiments.

Experiment 1. In this phase we evaluated the relationship be-tween systemic and epidural temperature after SCI and assessed the effects of modest systemic hypothermia on histopathological dam-age at 7 days post-SCI.

Experiment 2. In this phase we evaluated the effects of modest systemic hypothermia on locomotor function and histopathological damage at 44 days post-SCI.

Surgical Preparation

After induction of halothane anesthesia (4% in 70% N$_2$O, bal-anced with O$_2$), 36 female Sprague–Dawley rats (16 in Experiment 1 and 20 in Experiment 2) weighing 225 to 275 g had their necks and backs shaved and scrubbed with betadine. Using aseptic tech-niques, we created a midline cervical incision to expose the trachea. Under direct visual exposure, the rats were intubated using a No. 14 French endotracheal tube. The wound was closed using surgical clips. Anesthesia was continued with halothane (2% for insertion of vascular catheters, 0.5% for trauma, hypothermia/normothermia, and maintenance) via artificial ventilation at 60 cycles/minute and a tidal volume of 3 ml (depending on blood gas measurements). The antibiotic Crystiben (0.01 ml/100 g) was administered intramus-cularly to prevent iatrogenic infection. A catheter was surgically placed in the tail artery for withdrawal of blood, administration of saline, and continuous monitoring of MABP. An important aspect of the present study was the effort made to ensure constant physio-logical conditions for all animals. Physiological parameters in-cluding PO$_2$, PCO$_2$, and pH were maintained by adjusting the level of anesthesia, respiratory rate, and end-tidal volume, and they were measured with a blood gas analyzer (Radiometer, ABL 330, Copen-hagen, Denmark) every 2 hours in Experiment 1 and every hour in Experiment 2.

Contusion Spinal Cord Injury

Traumatic SCI was produced in Experiments 1 and 2 following a T-10 laminectomy. The animals were positioned in the weight-drop apparatus (NYU Impactor). Two spinal clamps were attached to the T8–9 and T11–12 spinal processes, respectively. A transducer was placed at the site of the muscle near the spinal column, and the impactor rod (10 g) was centered above T-10. The rod was slowly lowered until contact with the dura was made, which was deter-mined by completion of a circuit that resulted in an audible tone. The spinal cord was then contused with the NYU weight-drop device that released a 10-g rod from a height of 12.5 mm onto the exposed spinal cord. Impact analysis of data, including the degree of cord compression, velocity, duration of impact, and height of weight drop, were recorded by a preset NYU impactor software package.[27] After trauma was induced, the rats were randomized to hypothermia and normothermia treatment groups.

Hypothermia and Normothermia Treatment

Systemic temperature was controlled with a circulator water pump connected to a Plexiglas chamber with two thermal blankets. Each rat was placed in the chamber and a flexible thermistor was inserted in the rectum. Prior to induction of SCI, all animals were maintained at a (rectal) temperature of 37°C by using a feedback-controlled heating blanket. After induction of SCI, a small temper-ature probe placed at the site of laminectomy in the epidural space was used to monitor epidural temperature. Beginning 30 minutes posttrauma, the rats were placed in the Plexiglas environmental chamber to maintain rectal and epidural temperatures. For the nor-mothermia group (nine in Experiment 1 and eight in Experiment 2), the temperature was adjusted to maintain a temperature of 37.3 ± 0.1°C in Experiment 1 and 37 ± 0.1°C in Experiment 2 for 4 hours post-SCI. For the hypothermia rat group (seven in Experiment 1 and 12 in Experiment 2), the temperature was adjusted to maintain an epidural temperature of 33.1 ± 0.2°C in Experiment 1 and 32 ± 0.1°C in Experiment 2 for 4 hours. These differences in temperature were not significantly different and were within the range repre-senting moderate hypothermia. After each post-SCI treatment ses-sion, the rats were housed in cages containing soft bedding and treated with intramuscular injections of Crystiben every other day for 7 days. The rats were checked daily, and bladders were palpat-ed at least twice daily and emptied as required until they regained reflex voiding.

Behavioral Assessment of Rats in Experiment 2

Open-field locomotor function was evaluated by obtaining the BaBeBr locomotor score in animals in Experiment 2.[3] For a brief period preoperatively, rats in Experiment 2 were exposed daily for 1 week to the behavioral testing facility to acclimate them to open-field exploration. Each rat was tested for 4 minutes. Postoperative open-field testing of animals in Experiment 2 occurred twice a week starting on Day 2 postinjury and continuing to Day 44 postinjury. Examiners were blinded to the type of postinjury treatment received by each animal.

Histopathological Assessment

At 7 days (Experiment 1) and 44 days (Experiment 2) post-SCI, rats were deeply anesthetized with sodium pentobarbital and per-fused transcardially with isotonic saline for 5 minutes, followed by a mixture of 4% formaldehyde, glacial acetic acid, and methanol 1:1:8 by volume for 30 minutes. Spinal cords were removed and embedded in paraffin in 16 to 18 mm–long blocks that contained the contusion epicenter as well as noninjured tissue at the end of each block. Each block was serially sectioned (longitudinally) at 10 μm. Sections were stained with hematoxylin and eosin for histopatho-logical analysis. Sixteen sections obtained from the "central core" of the spinal cord were studied with light microscopy, and recon-structions of the longitudinal area of tissue damage in these sections

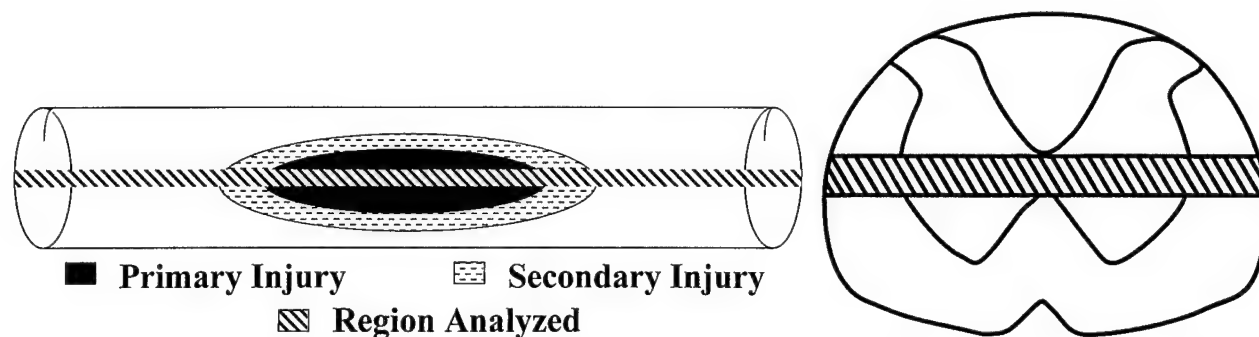■ **Primary Injury**   ▨ **Secondary Injury**
▨ **Region Analyzed**

FIG. 1. The location of sections sampled for the quantitative analysis of tissue damage resulting from weight-drop injury. Sixteen longitudinal sections from the middle of the spinal cord (*shaded area* in *upper*) were selected for analysis.   *Left:* Longitudinal representation of the spinal cord, showing the rostrocaudal extent of the primary *(black)* and secondary *(dashed lines)* injury as well as intact tissue at each end of the block. The area highlighted by the *diagonal lines* represents the location of sections sampled for analysis.   *Right:* Cross-sectional representation of the cord, showing the location of sections selected from the "central core" of the cord. Each section contained a representative sample of white and gray matter.

were made with the aid of an overhead projector. The area of tissue damage in each section was quantitatively determined using computer-aided image analysis. The investigator conducting this evaluation was blinded to the type of postinjury treatment received by the rat. The total area of tissue damage in the 16 sections analyzed was then computed. The method of selecting sections from the middle of the cord (Fig. 1) was used because: 1) the middle sections of the spinal cord were of high histological quality in contrast to the upper and lower sections of the cord, which were usually of poor quality due to the effects of the contusive injury and/or the presence of artifacts due to histological processing; 2) sampling the same number of sections (16) obtained in the center of the cord ensured consistency in the way the analysis was performed; and 3) the 16 sections analyzed contained varying proportions of white and gray matter, thereby providing a representative sampling of these two anatomical regions of the spinal cord.

Statistical Analysis

All data are expressed as a mean ± SEM and analyzed using a commercially available computer program (StatView). For each rat, BaBeBr scores were averaged to yield one score per test session. Physiological data, BaBeBr scores, and total area of damage were compared between the normothermia and hypothermia groups at the same time point by using one-way analysis of variance and Fisher's protected least-significant difference test. Differences were considered to be statistically significant at p < 0.05.

Results

Experiment 1

Physiological Data and Contusion Parameters. An important objective in this study was to maintain MABP, blood gas levels, and pH within normal physiological ranges throughout all experimental procedures. As shown in Table 1, there were no significant differences in these parameters observed at any of the time points evaluated. There were also no significant differences observed among any of the pre- and postcontusion parameters recorded prior to and during the weight-drop procedure (Table 2).

Relationship Between Epidural and Rectal Temperatures. The baseline rectal and epidural temperatures measured pre-SCI were 36.7 ± 0.36 and 34.5 ± 0.5°C, respectively. On average the epidural pre-SCI temperature was 2.2°C

less than rectal temperature. In the normothermic rat group, rectal and epidural temperatures were maintained at 37 ± 0.1°C and 37.3 ± 0.1°C, respectively, for a 4-hour period posttrauma. On average, the epidural temperature post-SCI was 0.3°C higher than the rectal temperature. The rectal and epidural temperatures in rats receiving hypothermic treatment were lowered to 32.2 ± 0.1°C and 33.1 ± 0.2°C, respectively, 30 minutes posttrauma for 4 hours. On average, the epidural temperature post-SCI recorded in this group was 0.9°C higher than the rectal temperature. The average difference between the two treatment groups was 4.8°C for rectal and 4.2°C for epidural temperatures.

Histopathological Assessment. A summary of the histopathological outcome of rats tested in Experiment 1 is shown in Figs. 2 and 3. Analysis of the results showed that there was significant tissue damage at 7 days after

TABLE 1

Physiological data in 16 rats in which spinal cord sections were obtained 7 days postinjury (Experiment 1)*

Parameter	Hypothermia	Normothermia	p Value†
pretrauma			
MABP	122.5 ± 2.5	120.0 ± 0.0	NS
pH	7.45 ± 0.02	7.43 ± 0.01	NS
PCO_2	37.2 ± 1.5	36.7 ± 1.6	NS
PO_2	205.0 ± 34.8	192.1 ± 18.1	NS
2 hrs posttrauma			
MABP	130.0 ± 10.0	123.8 ± 6.3	NS
pH	7.41 ± 0.03	7.37 ± 0.01	NS
PCO_2	39.5 ± 2.1	39.9 ± 1.1	NS
PO_2	176.8 ± 15.3	198.4 ± 12.5	NS
4 hrs posttrauma			
MABP	128.0 ± 12.0	122.4 ± 8.5	NS
pH	7.36 ± 0.03	7.37 ± 0.01	NS
PCO_2	44.0 ± 3.1	41.3 ± 1.0	NS
PO_2	222.5 ± 29.3	172.3 ± 12.5	NS

*Values are presented as the mean ± SEM. Abbreviation: NS = not significant.

†According to one-way analysis of variance and Fisher's protected least-significant difference test.

TABLE 2

Contusion parameters in 16 rats in which spinal cord sections were obtained 7 dats postinjury (Experiment 1)*

Parameter	Hypothermia	Normothermia	p Value†
compression	1.76 ± 0.12	1.81 ± 0.06	NS
height (mm)	12.6 ± 0.5	12.2 ± 0.2	NS
velocity (m/sec)	0.47 ± 0.02	0.46 ± 0.12	NS
duration of impact (msec)	52.0 ± 2.8	49.8 ± 1.6	NS

* Values are presented as the mean ± SEM.

†According to one-way analysis of variance and Fisher's protected least-significant difference test.

traumatic SCI in the normothermia-treated rats. In rats receiving hypothermic treatment significantly less tissue damage was demonstrated compared with the normothermia-treated group ($p < 0.01$; Fig. 2). The effects of hypothermia resulted in a 31.7% decrease in the total area of tissue damage compared with that found in the normothermia-treated group. An example of the histopathological difference between the normothermia- and hypothermia-treated groups is shown in sections shown in Fig. 3A and B.

Experiment 2

Physiological Data and Contusion Parameters. All physiological data including MABP, PCO_2, PO_2, and pH were maintained within normal ranges throughout all experimental procedures (Tables 3 and 4). It should to be noted that the MABP in the hypothermia-treated group (for the 4-hour treatment period) was higher than in the normothermia-treated group (99 ± 3.7 mm Hg and 78.8 ± 3.1 mm Hg, respectively, $p < 0.01$). No significant differences in contusion parameters were found between the two treatment groups (Table 4).

Behavioral Assessment. Immediately after SCI, rats demonstrated flaccid paralysis. In the normothermia-treated group few or no hindlimb movements were observed at 5 days posttrauma, and a gradual recovery was seen over the next 2 weeks. By 3 weeks posttrauma, most animals were stepping consistently but lacked forelimb–hindlimb coordination. There was no behavioral improvement in normothermia-treated animals between Weeks 3 to 6 posttrauma. Nine days after injury, locomotor performance scores in animals receiving hypothermic treatment were significantly increased over those exposed to normothermic treatment ($p = 0.0099$). This trend continued throughout the duration of the evaluation period (Fig. 4). On Day 44 after injury, the final mean BaBeBr score for hypothermia-treated animals was significantly higher than that of normothermia-treated animals (13.3 ± 0.47 and 10.8 ± 0.44, respectively; $p = 0.0024$; Fig. 5).

Histopathological Assessment. Analysis of the results showed that there was significant tissue loss at 44 days after SCI in the normothermia-treated group (Fig. 3C). Following treatment with hypothermia a cavitary lesion within the spinal cord was still observed (Fig. 3D), but the total area of damage was significantly less than that in rats exposed to normothermia at 44 days postinjury ($p < 0.01$, Fig. 6). There was a 15.8% difference between the rats receiving hypothermic and normothermic treatment. Examples of sec-
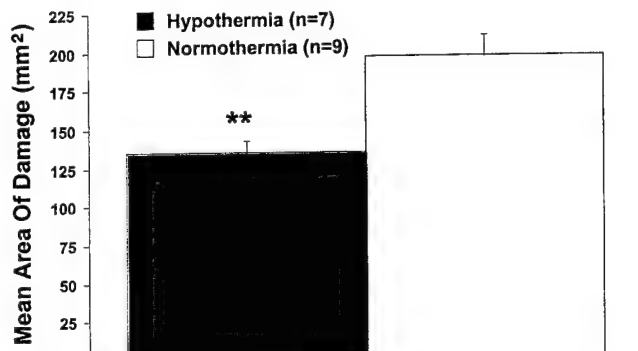
AREA OF DAMAGE AT DAY 7

FIG. 2. Bar graph showing the area of tissue damage following hypothermia or normothermia treatment. The hypothermia-treated rats received treatment (32–33°C) 30 minutes after trauma for 4 hours. Normothermia-treated rats received treatment (37°C) 30 minutes after trauma for 4 hours. Data are presented as mean ± standard error of the mean. **$p < 0.01$.

tions obtained in animals in the normothermia- compared with hypothermia-treated groups are shown in Fig. 3C and D, respectively.

Discussion

The results of this study have shown that the NYU weight-drop device[4,27] produces reliable contusion injuries with reproducible anatomical characteristics and behavioral deficits. Our results support the conclusions drawn in previous studies that this model is useful in testing the preclinical efficacy of therapeutic strategies for clinical application. In the present study, we have demonstrated the beneficial effects that are conferred by modest systemic hypothermia delivered for a period of 4 hours after injury on functional and morphological outcome measures in the rat.

Epidural Temperature and Systemic Hypothermia

In this study epidural temperature was effectively lowered (32–33°C) by whole-body cooling. Several approaches have been used to lower spinal cord temperature, including profound whole-body hypothermia, selective cooling of the epidural or intrathecal space, or perfusion of the spinal cord.[36] Profound whole-body hypothermia treatment has several potential complications including the induction of undesirable changes in clotting, pulmonary, or cardiovascular function.[18] On the other hand, LSCC has been shown to be effective in the lowering of spinal temperature and in the treatment of experimental SCI.[8,22,23,31,48] Similar beneficial results have been reported in some clinical studies, but LSCC studies are difficult to evaluate because they often include relatively few patients and lack randomized control groups.[37,50] In most experimental and clinical studies in which investigators use LSCC, temperature was significantly lowered (by approximately 10°C), but the clinical application of this technique has many technical difficulties, requiring special cooling devices, emergency surgery, and complicated postoperative management.[30,50] Additionally,
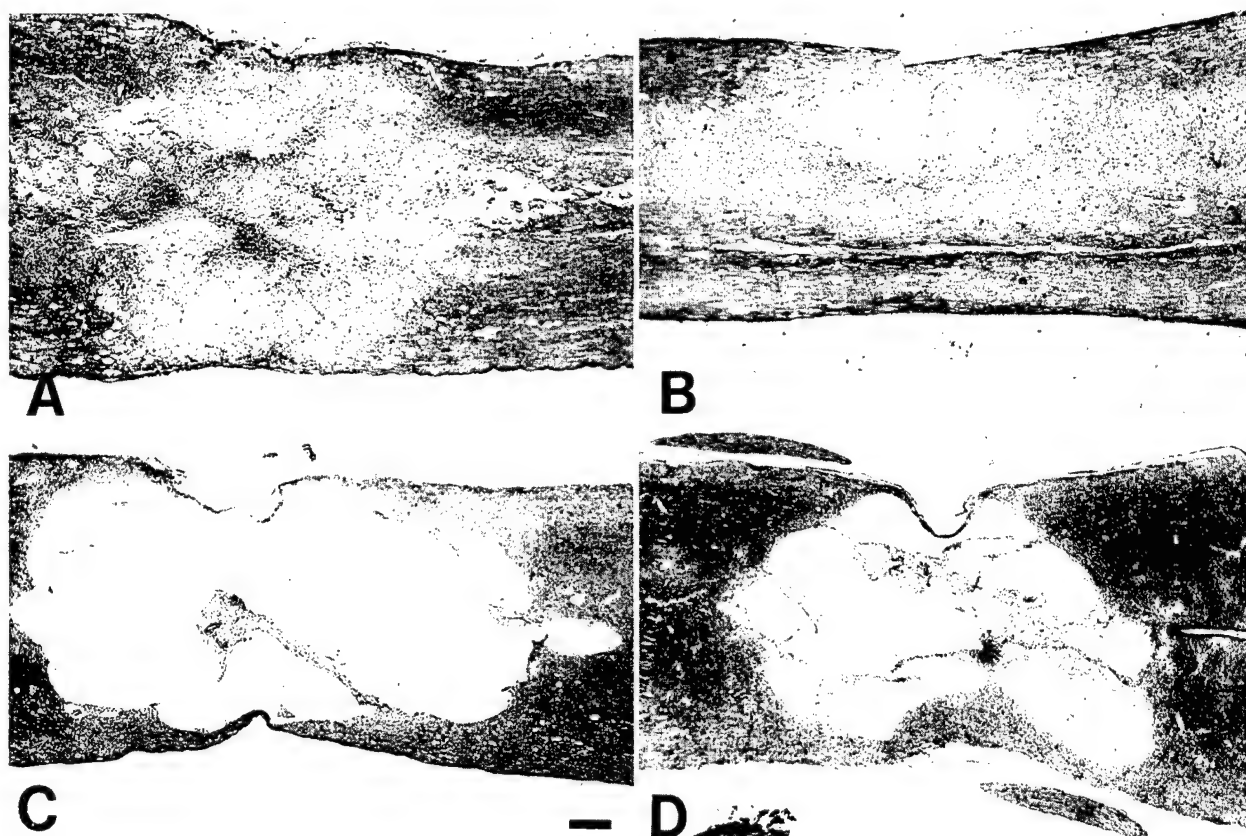
FIG. 3. Photomicrographs of histological sections obtained in the spinal cords of animals that received contusion injury. Tissue damage in normothermic animals is shown at 1 week (A) and 6 weeks (C) following injury. Treatment with modest hypothermia starting 30 minutes after moderate traumatic injury for 4 hours reduced the lesion area at 1 week (B) and 6 weeks (D). Note the reduced area of tissue damage (B) and size of cavity (D) following hypothermia. H & E, original magnification × 25.

the high mortality rates reported in some studies are a major concern for clinical application.

Previous findings related to the neuroprotective effects of moderate systemic hypothermia (30–33°C) following brain ischemia and trauma may be applicable to SCI.[20] An important question, however, is whether modest temperature changes in the spinal cord can be produced via systemic hypothermia. For this reason it was important to determine the relationship between systemic (rectal) and epidural temperatures. In the present study, the epidural temperature recorded in the hypothermic group was approximately 1° (0.9°C) higher after SCI, compared with systemic (rectal) temperature in the same group. In the normothermia-treated group, however, this difference was only 0.3°C higher than the systemic temperature. These data suggest that systemic temperature is a close approximation of spinal cord temperature and that application of systemic hypothermia is an effective and simple strategy for lowering spinal cord temperature.

Histopathological Outcome of Modest Systemic Hypothermia

The morphological results of the present study showed that modest hypothermia has neuroprotective effects on histopathological damage following contusion-induced SCI. The morphological changes associated with traumat-

ic SCI in the rat have been well characterized.[5,13,42,43] Our results have shown that following normothermic treatment, there was significant tissue damage that included hemorrhagic necrosis, cell loss, axonal swelling, and vacuolization, particularly in the gray matter, at both 1 week and 6 weeks post-SCI. The acute damage observed at the injury epicenter was located centrally in the spinal cord but progressed outwardly and rostrocaudally to involve adjacent areas of gray and white matter.[5,42] In the hypothermia-treated group, sparing of gray and white matter occurred at both 7 days and 44 days post-SCI, and the rostrocaudal extent of total tissue damage was significantly reduced. Although no efforts were made to distinguish between the protective effects of hypothermia on white compared with gray matter, the effects observed on locomotor function, as reflected by BaBeBr scores, suggest that hypothermic treatment had a significant effect on white matter regions of the spinal cord.

To establish a meaningful relationship between hypothermia and normothermia treatment groups as well as to determine the morphological correlate for the behavioral improvement observed in rats receiving hypothermia treatment, it was imperative to quantify the amount of tissue damage in animals undergoing different post-SCI therapies. Several investigators have developed methods by which to quantify the amount of tissue damage follow-

TABLE 3

*Physiological data in 20 rats in which spinal cord sections were obtained 44 days postinjury (Experiment 2)**

Variable	Hypothermia	Normothermia	p Value†
pretrauma			
epidural temp	34.5 ± 0.3	35.0 ± 0.2	NS
rectal temp	36.7 ± 0.1	36.7 ± 0.1	NS
MABP	95.6 ± 2.9	86.7 ± 4.7	NS
pH	7.41 ± 0.01	7.41 ± 0.01	NS
PCO_2	42.7 ± 1.3	40.5 ± 1.4	NS
PO_2	139.6 ± 6.1	139.7 ± 17.1	NS
1 hr posttrauma			
epidural temp	32.0 ± 0.1	37.1 ± 0.1	<0.001
rectal temp	31.6 ± 0.1	37.0 ± 0.1	<0.001
MABP	100.0 ± 3.5	82.2 ± 4.3	<0.005
pH	7.42 ± 0.02	7.42 ± 0.01	NS
PCO_2	37.5 ± 1.4	39.5 ± 1.4	NS
PO_2	149.7 ± 9.6	139.3 ± 11.7	NS
2 hr posttrauma			
epidural temp	32.0 ± 0.1	37.0 ± 0.1	<0.001
rectal temp	31.6 ± 0.2	37.0 ± 0.1	<0.001
MABP	100.3 ± 3.3	75.6 ± 2.4	<0.001
pH	7.41 ± 0.01	7.42 ± 0.01	NS
PCO_2	38.9 ± 1.0	37.8 ± 1.7	NS
PO_2	140.4 ± 9.2	148.4 ± 11.1	NS
3 hr posttrauma			
epidural temp	31.9 ± 0.1	37.0 ± 0.0	<0.001
rectal temp	31.5 ± 0.1	37.0 ± 0.1	<0.001
MABP	99.4 ± 3.6	80.0 ± 2.9	<0.01
pH	7.41 ± 0.01	7.40 ± 0.01	NS
PCO_2	38.2 ± 1.1	39.8 ± 1.2	NS
PO_2	138.3 ± 8.6	150.9 ± 8.8	NS
4 hr posttrauma			
epidural temp	32.0 ± 0.1	37.0 ± 0.1	<0.001
rectal temp	31.7 ± 0.1	36.9 ± 0.1	<0.001
MABP	96.3 ± 4.1	77.2 ± 2.8	<0.01
pH	7.41 ± 0.01	7.40 ± 0.01	NS
PCO_2	37.7 ± 0.8	39.9 ± 0.8	NS
PO_2	144.3 ± 9.5	152.1 ± 8.3	NS

*Values are presented as the mean ± SEM. Abbreviation: temp = temperature.

†According to one-way analysis of variance and Fisher's protected least-significant difference test.

TABLE 4

*Contusion parameters measured in 20 rats in which spinal cord sections were obtained 44 days postinjury (Experiment 2)**

Parameter	Hypothermia	Normothermia	p Value†
compression	1.62 ± 0.05	1.57 ± 0.06	NS
height (mm)	12. ± 0.1 12.3 ± 0.1 NS		
velocity (m/sec)	0.4 ± 0.01 0.49 ± 0.01		NS
time (msec)	46.8 ± 1.2 47.6 ± 1.6 NS		

*Values are presented as the mean ± SEM. Abbreviation: NS = not significant.

†According to one-way analysis of variance and Fisher's protected least-significant difference test.

aspartate antagonist and inducible nitric oxide synthase inhibitor, on morphological and functional outcome following SCI.[52]

Behavioral Recovery and Modest Systemic Hypothermia

The behavioral results obtained in rats in the present study demonstrated that the time course of locomotor recovery in the normothermia-treated group was similar to that found in previous studies in the rat following moderate injury.[4,44] The BaBeBr locomotor score used in this study is a multiple function test of motor outcome that provides an efficient, expanded, and unambiguous locomotor rating.[3,4] The BaBeBr scores differ from other motor scoring systems in several respects. First, the score is not a summation of component behaviors. Each BaBeBr score requires fulfillment of a unique set of criteria. Second, the scores encompass many behavioral traits and represent a detailed characterization of rat locomotor function. In this study, 1 week after injury, ratings of motor performance in rats receiving hypothermia treatment were significantly increased over those exposed to normothermia treatment. This trend continued throughout the duration of the study, and the dif-

ing SCI including: 1) lesion volume[12,13] and 2) percentage of spared spinal cord tissue.[5,34,38] In the present study we used a histopathological method that relied on the selection of 16 longitudinal sections from the middle of the spinal cord. There are several major advantages to this approach: the selected sections from the "central core" of the cord included representative regions of white and gray matter; the use of longitudinal sections make it possible to visualize the full rostrocaudal extent of the injury; the central cord region is typically the region affected most by contusion injury of the cord; and the middle sections of the cord were of high quality. Using the central core method of analysis our results support the conclusion that modest systemic hypothermia has beneficial effects on histopathological damage following contusion-induced SCI. The mean sum of damaged areas demonstrated in hypothermia-treated animals were significantly less than those in the normothermia-treated group at both 1 week and 6 weeks post-SCI. Additional validation of this technique derives from studies in our laboratory that have shown the beneficial effects of agmatine, an N-methyl-D-

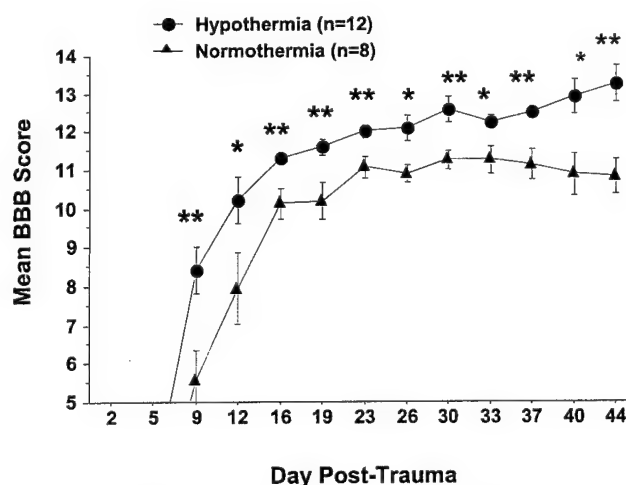FIG. 4. Graph showing time course of locomotor recovery as measured by BaBeBr scores following hypothermic and normothermic treatment. Mean BaBeBr scores obtained in animals receiving modest hypothermia (32–33°C) 30 minutes after trauma for 4 hours are represented by the *filled circles* and normothermia (37°C) are represented by *triangles*. Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

B-B-B SCORE AT DAY 44
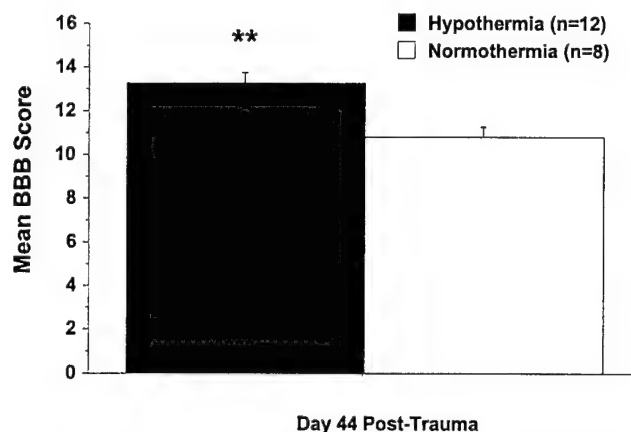

Day 44 Post-Trauma

FIG. 5. Bar graph illustrating the final BaBeBr scores following normothermic and hypothermic treatment. The hypothermia group received treatment (32–33°C) 30 minutes after trauma for 4 hours. The normothermic group received normothermia (37°C) 30 minutes after for 4 hours. Data are represented as mean ± SEM. There was a significant difference between the final BaBeBr scores following hypothermic as compared with normothermic treatment. **p < 0.01.

AREA OF DAMAGE AT DAY 44
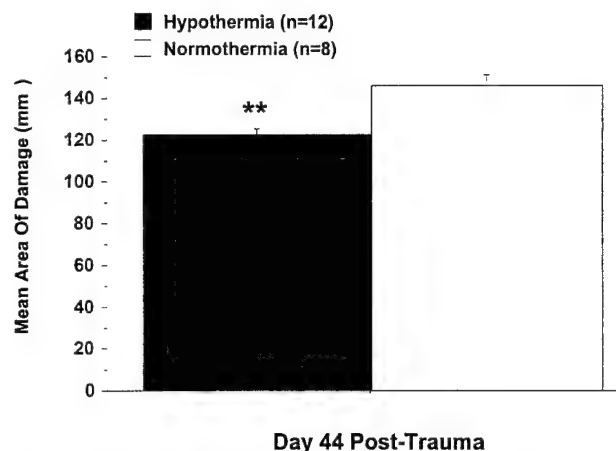

Day 44 Post-Trauma

FIG. 6. Bar graph depicting the area of damage following weight-drop injury and treatment with hypothermia or normothermia. The hypothermia-treated rats received treatment (32–33°C) 30 minutes after trauma for 4 hours. The normothermia-treated rats received treatment (37°C) 30 minutes after trauma for 4 hours. Data are represented as mean ± SEM. There was a significant difference in the final area for the two treatment groups. **p < 0.01.

ference became larger over the 6-week evaluation period. On Day 44 after injury, the final mean BaBeBr score for animals in the hypothermia-treated group was significantly higher than that in normothermia-treated rats. These results are consistent with those reported in previous studies in which application of systemic hypothermia was shown to be protective against brain and spinal cord ischemia caused by vascular occlusion.[36] To our knowledge, this is the first systematic evaluation in which the beneficial effects of modest (32–33°C), systemic hypothermia are shown on morphological and behavioral outcomes following traumatic SCI.

Mechanisms of Modest Systemic Hypothermia-Mediated Protection

The mechanisms of hypothermia-mediated protection against histopathological damage and locomotor function following contusion-induced SCI are not understood. Several possibilities have been suggested.[21] Early speculation was based on the reduction in tissue metabolic and oxygen requirements that occurs when CNS tissue is cooled.[37] Since the mid-1950s and late 1970s the application of hypothermia has been demonstrated to protect against the loss of phosphocreatinine, accumulation of lactate, and decrease of cerebral oxygen consumption as well as lowering the rate of cerebral adenosine triphosphate depletion following the interruption of cerebral circulation.[28,29,33,39,46] During the 1980s and 1990s, experimental studies and clinical observations showed that brain and spinal cord lesions are greatly enlarged by secondary injury.

Although the molecular and cellular mechanisms underlying secondary injury are still not clearly understood, evidence suggests that hypothermia influences several

changes responsible for this component of injury. For example, hypothermia attenuates the production of oxygen free radicals,[25] suppresses release of the excitatory amino acid neurotransmitter glutamate,[25,36] reduces intracellular calcium overload,[2] prevents loss of microtubule-associated protein–2[47] and the delayed induction of inducible nitric oxide synthase,[15] diminishes induction of interleukin-1β messenger RNA,[26] and inhibits inflammatory responses[51] as well as lowers lipid peroxidation.[49] In a recent SCI study we have shown that another potential contributing factor to the effects of hypothermia is the reduction of posttraumatic inflammation.[16] In this study we observed a significant reduction in the accumulation of polymorphonuclear leukocytes following hypothermic treatment. In addition, various investigators have demonstrated significant reductions in spinal cord blood flow following traumatic SCI.[37] Cooling the spinal cord could therefore have an effect on the degree of posttraumatic hypoperfusion.[37] In the present study, hypothermia-treated rats had a higher MABP than normothermia-treated rats. Similar results have been reported in the study by Mansfield and colleagues.[35] The increased MABP may have improved spinal cord blood flow in injuried regions with impaired autoregulation, thereby reducing ischemia in the early posttrauma period.[35]

Although the mechanism for the beneficial effects of hypothermia are not entirely understood, the fact remains that this intervention has a significant impact on the pathological and functional state of injured CNS tissue. To understand further the full scope of effects provided by hypothermia it will be important in the future to determine whether increasing the duration of postinjury hypothermia treatment offers any additional beneficial effects and whe-

ther it is possible to enhance the pharmacological effects or widen the therapeutic window of interventions by using such agents as methylprednisolone[9] or interleukin-10.[7] If the effects of immediate post-SCI hypothermia include broadening the therapeutic window for other interventions, this could provide an important benefit for patients in whom immediate surgical intervention is not possible. However, before modest systemic hypothermia can be used clinically, it will be important to determine the therapeutic window, optimal duration of hypothermic treatment post-SCI, and the ability of hypothermia to increase therapeutic efficacy of currently used agents such as treatment with methylprednisolone.

Conclusions

It is concluded that: 1) modest temperature changes in the spinal cord can be produced via systemic hypothermia; 2) modest systemic hypothermia (32–33°C) significantly protects against behavioral deficits following traumatic SCI; and 3) modest systemic hypothermia significantly reduces the area of tissue damage at both 7 and 44 days post-injury. The results lend support for the continued investigation into the therapeutic benefits of hypothermia delivered at different time points, for different durations, and in combination with pharmacological agents following injury.

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Neuroprotective Effects of Basic Fibroblast Growth Factor Following Spinal Cord Contusion Injury in the Rat

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ABSTRACT

Cytokines and neurotrophic factors have been implicated in the pathophysiology of injury to the central nervous system. While some cytokines are considered pro-inflammatory, other factors promote neuronal growth and survival. The present study investigated the neuroprotective effects of interleukins 1 (IL-1), 4 (IL-4), and 6 (IL-6), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor (bFGF) in a contusion model of spinal cord injury. Female Sprague-Dawley rats ($n = 55$) sustained a 10-g weight-drop injury to the lower thoracic spinal cord (T10) from a height of 12.5 mm using the NYU impactor. A micro-infusion system (Alzet minipump) was used to continuously deliver drugs or vehicle directly into the epicenter of the contused spinal cord starting 1 or three h postinjury. At the end of 7 days, animals were perfused and the cords removed for histopathological analysis. Longitudinal serial sections were cut on a freezing microtome and stained with cresyl violet. Areas of central necrosis, partial preservation, and total zone of tissue injury were identified and traced by an independent reviewer using a computer based imaging system. The mean total zone of injury in five animals receiving vehicle infusion was 18.04 ± 4.20 mm^3. The mean zone of partial preservation in these animals was 16.46 ± 3.32 mm. Basic fibroblast growth factor reduced the total zone of injury by 33% [$p < 0.01$, least significant difference (LSD) of Fisher] in five animals and the zone of partial preservation by 32% ($p < 0.01$, LSD of Fisher) when compared to controls. There were trends toward reduction in total zone of injury and zone of partial preservation in rats treated with IL-4, CNTF, and NGF versus vehicle; however, none of these reached statistical significance. No significant differences were observed between animals receiving vehicle versus bFGF treatment commencing 3 h after injury. These data demonstrate that the continuous intramedullary infusion of bFGF initiated one hour after moderate contusion injury of the spinal cord significantly reduces the total zone of injury and the zone of partial preservation. These results support the further investigation and possible future clinical application of bFGF in the treatment of acute spinal cord contusion injury.

Key words: ciliary neurotrophic factor; cytokine; interleukin-4; nerve growth factor; neurotrophic factor

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INTRODUCTION

THE INITIAL TRAUMA INDUCED BY INJURY together with a complex cascade of events following injury determine the degree of total tissue damage and the ultimate neurological outcome following spinal cord injury (SCI). Presently, numerous agents are proposed to be neuroprotective against central nervous system (CNS) injury (for recent reviews, see McIntosh, 1993; Mattson and Scheff, 1994; Mocchetti and Wrathall, 1995). Steroids, neurotrophins, cytokines, and gangliosides have been demonstrated to promote neuronal survival or support neuronal growth in various *in vivo* systems (Mattson and Scheff, 1994; Blottner and Baumbarten, 1994; Olson et al., 1994). Methylprednisolone improves neurological recovery when given early after human spinal cord injury (Bracken et al., 1990). Recently, neurotrophins have also been used in several disease models: glial cell-derived neurotrophic (GDNF) in Parkinson's disease, nerve growth factor (NFG), and ciliary neurotrophic factor (CNTF) in Alzheimer's disease, and insulin-like growth factor (IGF-1) in multiple sclerosis (Hefti, 1997). The neurotrophic factor basic fibroblast growth factor (bFGF) has also been reported to be neuroprotective in models of cerebral ischemia and traumatic brain injury (Koketsu et al., 1994; Fisher et al., 1995; Dietrich et al., 1996; Ay and Finklestein, 1998) and to protect neurons from axotomy-induced death (Peterson et al., 1996). In a model of spinal cord compression, bFGF administered locally at the site of lesion was reported to improve hindlimb function in combination with methylprednisolone infusion (Baffour et al., 1995). Recently Teng et al. (1997) reported basic and acidic fibroblast growth factor (FGF) to be neuroprotective for cholinergic neurons following confusion injury in the rat.

Interleukin 4 (IL-4) has been shown to be an anti-inflammatory cytokine, regulating neutrophil and monocyte/macrophage functions (Luering et al., 1997; Niiro et al., 1997). Although the clinical significance has not been established, increased levels of IL-6 have been reported in the cerebrospinal fluid of head injury patients (Relton et al., 1997). IL-1, a pro-inflammatory cytokine, was found to exacerbate ischemic brain injury (Relton et al., 1997). In the study of spinal cord trauma, experimental studies are needed to assess the consequences of neurotrophic growth factor and cytokine treatment on histopathological outcome.

The purpose of the present study was to determine the effects of various cytokines and neurotrophic factors on histopathological outcome using a well characterized weight-drop device to produce spinal cord trauma. In this preliminary examination of different agents we utilized a continuous intramedullary infusion system to reliably deliver various factors directly into the site of injury. Our results indicate that the intramedullary infusion of bFGF initiated one hour after moderate SCI significantly reduces two measures of tissue damage, including the zone of partial preservation and the total zone of injury.

MATERIALS AND METHODS

Model of Injury

Adult female Sprague Dawley rats weighing 250–325 g were used in this study. All procedures were approved by the University of Miami Animal Care and Use Committee. Inhalational anesthesia was provided with a balanced halothane, NO_2 and O_2 mixture. Local anesthetic of 0.25% lidocaine with 1:400,000 epinephrine was used to infiltrate the skin and paraspinous muscles. A single dose of intramuscular antibiotic (50 mg/kg of cefozalin) was administered at the beginning of the procedure. Aseptic techniques were employed to perform a one level complete laminectomy with bilateral medial facetectomy at the lower thoracic (T10) level of the cord. A 10-g weight drop utilizing the NYU impactor from 12.5 mm was performed while monitoring start time, height, and velocity curves. Weight drops with less than 5% height and velocity errors, as well as resultant bilateral cord hematoma and immediate postoperative paraplegia were used as inclusion criteria. Rectal temperature was monitored and isothermic blankets utilized to maintain core temperature at 37°C during the surgical procedure.

Cytokines/Growth Factors

All drugs were purchased from Genzyme Corporation (Cambridge, MA), including IL-1, IL-4, IL-6, bFGF, NGF, and CNTF. Solvents and dilution factors used to prepare each drug for infusion are listed in Table 1. Two assumptions were made in the calculation of infused drug concentration to ensure adequate drug delivery to the different zones of injury: (1) the cord is approximately a cylinder ($V = \pi r^2 h$) with an average cord diameter of 0.4 cm at the T10 level; and (2) there is a 1:10 dilutional effect by cerebrospinal fluid. In preliminary experiments it was determined with the infusion of 2% Evans Blue for 7 days after weight drop (data not shown) that the longitudinal lesion length for the injury used in the present study was 0.8 cm. Based on this determination the total cord volume to be infused was approximately 0.1 cc^3. The amount of drug calculated for infusion was delivered every day until sacrifice. A 2–10 times excess (based on previously tested *in vitro* concentrations reported by Gen-

TABLE 1. DOSAGE OF INTERLEUKINS AND GROWTH FACTORS

Factor	Vial	Sp activity	Solvent	Dil	[Final]	Exc	Lit.
IL-1$_a$	25 μg/ml	8 $\times$ 10^6 μ/mg	PBS + 0.1% BSA	1:100	2 $\times$ 10^3 μ/ml	5$\times$	10 U/ml
IL-4	1.5 μg/ml	5 $\times$ 10^8 μ/mg	PBS + 1% BSA	No	1.5 $\times$ 10^4 μ/ml	2$\times$	200 U/ml
IL-6	5 μg/ml	5 $\times$ 10^8 μ/mg	PBS + 0.1% BSA	1:20	1.25 $\times$ 10^5 μ/ml	6$\times$	500 U/ml
NGF	20 μg/ml	5 $\times$ 10^5 μ/mg	H$_2$O	No	2 $\times$ 10^4 ng/ml	2$\times$	100 ng/ml
CNTF	100 μg/ml	2 $\times$ 10^7 μ/mg	PBS + 0.1% BSA	1:20	5 $\times$ 10^3 ng/ml	12$\times$	10 ng/ml
bFGF	1 mg/ml	N/A	0.1% CHAPS 0.5% BSA	1:500	10^4 ng/ml	2$\times$	20 ng/ml

The recommended *in vitro* concentrations were published by Genzyme Corporation (Seattle, WA).

IL, interleukin; NGF, nerve growth factor; CNTF, ciliary neurotrophic factor; bFGF, basic fibroblast growth factor.

zyme Corporation) infusion was achieved for each factor (Table 1). For the purpose of evaluating the vehicle ffect of different solvents used with each factor, we choose a solution of saline and 0.1 M PBS with 0.5% BSA for infusion in vehicle-treated animals. No efforts were made to evaluate the effects of H$_2$O or 0.1% CHAPS + 0.5% BSA in control animals.

Drug Delivery

An osmotically-driven infusion pump (Alzet minipump, Alza Pharmaceuticals, Palo Alto, CA) was used for continuous infusion of drugs. The pump was connected with PE10/50 tubing to a 30-gauge 3/4-inch needle to deliver drugs through a small dural and pial opening at the site of injury. The needle was advanced 2 mm near the midline at the site of contusion. Needle insertion and infusion of drugs were started 1 h postinjury, and continued at 1 μl/h for 7 days. The Alzet minipump was pre-primed and loaded with drugs, and filled to 225 μl, including the extra tubing (5 cm). The injection cannula was secured to the preserved lamina below the level of injury, and the pump was secured in a subcutaneous pocket with sutures.

Assessment of Injury and Protection

Animals were perfused with 10% formalin on day 7 under sodium pentobarbital anesthesia. After fixation, cords were removed and placed in 10% sucrose for 24 h. Cords were cut longitudinally (100 μm) on a freezing microtome and stained with cresyl violet. Two-dimensional mapping of the injury site was carried out. Area values were then used to calculate the volume of injury in each experimental group by numeric integration of sequential areas. In preliminary experiments designed to evaluate the reproducibility of the injury protocol, the NYU impactor was shown to produce a reproducible area of spinal cord contusion at 7 days after injury. The well demarcated injury site consisted of an area of central necrosis (CN) defined as the area of cystic degeneration and complete tissue necrosis (Fig. 1). Surrounding the area of CN was commonly a zone that appeared partially preserved. This zone of partial preservation (ZPP) was defined as the region of vacuolation, selective neuronal injury, and white matter swelling (Fig. 1). The present three-zone injury model was established to recognize the total impact of the injury (TZI), the irreversible zone of injury (CN), and the intermediate injury zone, which could potentially
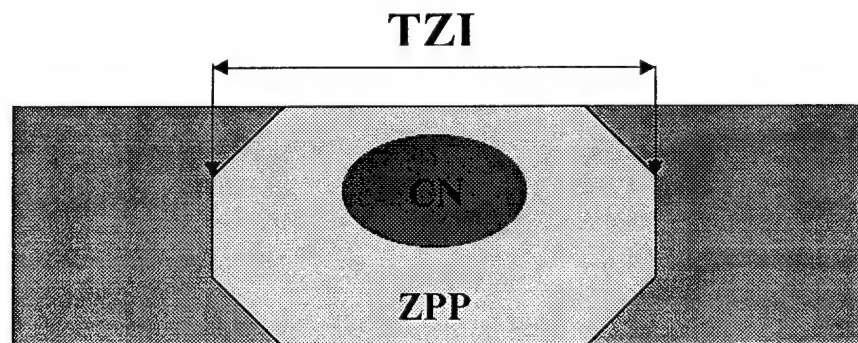
FIG. 1. Schematic illustration of the spinal cord contusion injury caused by the NYU impactor. TZI, total zone of injury; ZPP, zone of partial preservation; CN, central necrosis. Central necrosis was defined as the area of cystic degeneration and tissue necrosis. The zone of partial preservation was defined as the region of vacuolation, selective neuronal injury, and parenchymal edema, though the neuropil was intact and was calculated as the difference between TZI and CN.

be rescued and reduced with treatment (ZPP). In this study, both the area of central necrosis and total zone of injury (TZI) were measured by the same independent blinded reviewer. The zone of partial preservation was calculated as the difference between TZI and CN (Fig. 1).

Experimental and vehicle-treated groups consisted of seven to eight rats. In all groups, except the IL-1–treated animals, no more than one animal died prior to perfusion in each group. In the IL-1–treated group, five of 12 animals died prior to perfusion. The cords of seven animals were processed in each group, and the two animals with the highest and lowest injury volumes (TZI) were excluded from analysis. Experimental groups consisting of five animals were compared with vehicle-treated SCI animals ($n = 5$). The data were analyzed first with the Shapiro-Wilk test to confirm the normal distribution of each data set (Shapiro and Wilk, 1965). The results of this test dictated the use of parametric statistics, and, therefore, a one-way analysis of variance (ANOVA) was used to determine if there were significant differences among the different experimental groups. To determine which groups were different, we then performed the least significant difference procedure of Fisher (Daniel, 1995). The volume of tissue damage, i.e., TZI and ZPP, are presented as mean $\pm$ standard error, and p values of <0.05 were considered significant.

RESULTS

In the vehicle-treated group ($n = 5$), the mean volume of TZI was 18.04 ± 4.20 mm^3 and the mean ZPP was 16.46 ± 3.32 mm^3. The mean injury volumes of different treatment groups are listed in Table 2. Animals receiving IL-1 were found, on the average, to have larger TZI and ZPP volumes compared to control animals, though the difference was not significant ($p > 0.05$). The animal survival rate was also lower (7/12) in the IL-1–treated group. IL-6 did not have any appreciable ef-

fects on any of the injury parameters (Figs. 2 and 3). By contrast, bFGF was the most effective agent in reducing both the TZI ($p < 0.01$, LSD of Fisher), and ZPP ($p < 0.01$, LSD of Fisher) (Figs. 2 and 3). Overall, bFGF reduced TZI by 33% and ZPP by 32% compared to control animals. The effect on central necrosis was more variable, and not statistically significant (Table 2).

Though not statistically significant, three other drugs were also found to have effects on the volume of contusion injury. CNTF, NFG, and IL-4 reduced TZI (LSD of Fisher: CNTF, $p = 0.10$; NGF, $p = 0.13$; IL-4, $p = 0.12$). CNTF infusion had the highest percentage reduction (23%) of these three factors on TZI (IL-4, 22%; NGF, 21%). Of these three factors, IL-4 reduced ZPP by 20% below control level, CNTF (17%). None of the drugs tested reduced central necrosis to a statistically significant level because of variability and the relatively small volumes of central necrosis, though CNTF, IL-6, and NGF did show a sizable mean percentage reduction of over 80% (Table 2). Because of the small sample size and variability in the model, it is also possible that these agents are not effective.

In an effort to evaluate the potential therapeutic window of drug infusion, bFGF as the most effective treatment was selected for delivery 3 h postinjury. The comparison of this treatment with vehicle infusion showed no significant reduction in either TZI or ZPP (Fig. 4; $p > 0.05$, LSD of Fisher).

DISCUSSION

The CNS response to injury uniquely involves the interactions between multiple cell types and injury processes (Hefti, 1997; Hirschberg et al., 1994; Relton et al., 1997). Cytokines and neurotrophic factors have been reported to promote neural growth and protect against ischemic, traumatic, and chemically induced neuronal damage. These intercellular factors act primarily in a

TABLE 2. EFFECT OF CYTOKINES AND GROWTH FACTORS ON SPINAL CORD CONTUSION INJURY

| Drug infusion	Total no. of rats	No. of rats survived	No. of rats for analysis	Total zone of injury	Central necrosis	Zone of partial preservation
Control	7	7	5	18.04 ± 4.20 mm^3	1.57 ± 2.08 mm^3	16.46 ± 3.32 mm^3
IL-6	12	7	5	18.03 ± 2.66 mm^3	0.42 ± 0.37 mm^3	17.61 ± 2.86 mm^3
IL-1	7	7	5	21.43 ± 6.46 mm^3	1.01 ± 1.93 mm^3	20.42 ± 6.67 mm^3
IL-4	7	7	5	14.07 ± 2.03 mm^3	0.89 ± 1.08 mm^3	13.18 ± 3.04 mm^3
bFGF	7	7	5	12.05 ± 3.08 mm^3	1.09 ± 0.58 mm^3	11.17 ± 2.70 mm^3
NGF	7	7	5	14.20 ± 4.60 mm^3	0.89 ± 1.45 mm^3	13.23 ± 3.72 mm^3
CNTF	7	7	5	13.86 ± 2.45 mm^3	0.22 ± 0.28 mm^3	13.64 ± 2.22 mm^3

ZPP was calculated as TZI − CN.
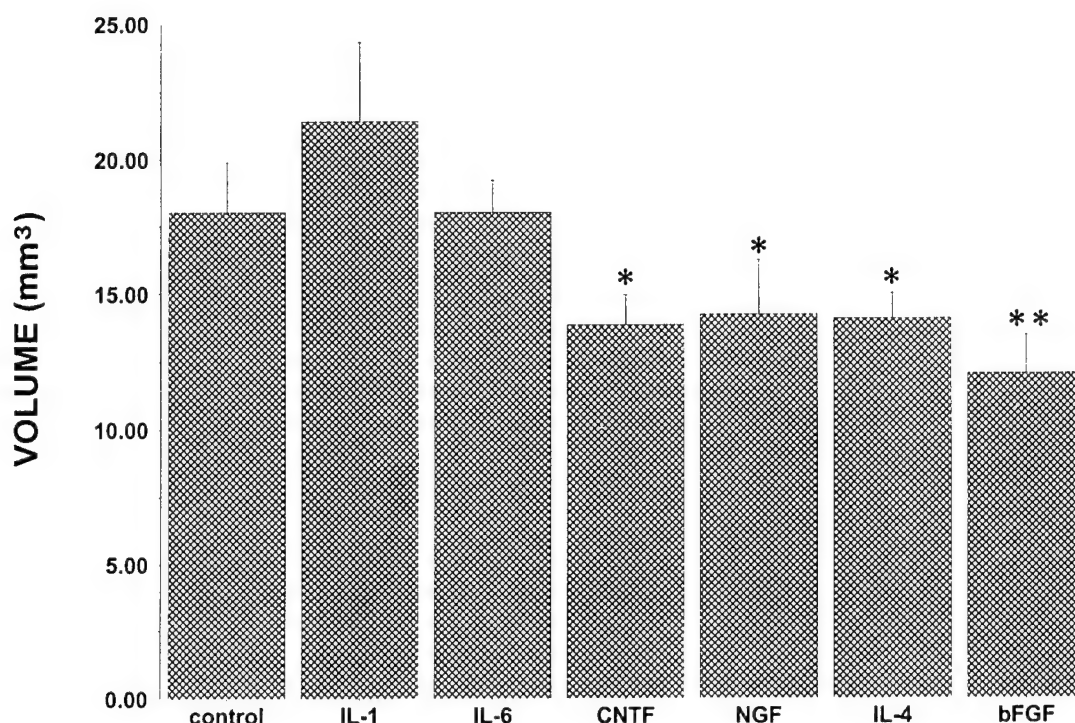
FIG. 2. Effects of drug infusion commencing 1 h postinjury and continuing for 7 days on the mean volume for the total zone of injury (TZI). Compared to infusion of vehicle (control), IL-1 was found to have a detrimental effect, IL-6 no effect, while bFGF significantly reduced the TZI. Although the effects of CNTF, NGF, and IL-4 were not significant, they did produce a reduction in the volume of TZI. $**p < 0.01$.

paracrine fashion, with glial and inflammatory cells producing and secreting them locally (Maeda, 1994). The posttraumatic breakdown of the blood-brain-barrier (BBB) also permits the extravasation of blood-borne factors and cellular elements that would be expected to promote inflammatory processes. Thus, two primary factors may potentially act against each other following the initial damage of the central nervous system: (a) inflammatory factors that exacerbate damage and (b) the cytokines/neurotrophic factors that promote neuronal regeneration and recovery (Giulian et al., 1988). Certain cytokines may cause further tissue damage by the induction of a surface mitogen mediated immune response, as well as by direct cytotoxicity (Birdsall, 1991). It must be stressed that following injury to the brain or spinal cord, multiple factors affect the cellular response to injury, leading to complex interactions that may affect outcome. Thus, although the use of *in vitro* purified cellular systems are a powerful approach in which to investigate the cellular response to injury, experimental models of CNS injury are necessary to determine the potential use of these agents in a clinical setting.

The weight drop system, utilizing the NYU impactor, provides a well characterized model for spinal cord injury. Most spinal cord injuries are blunt in nature, caus-

ing parenchymal contusions, hematoma, and edema. In the present study, evidence of a well-defined contusion was seen at seven days after injury. The injury volumes used in the present study consisted of the TZI, ZPP, and CN. This designation distinguishes between the areas of partial injury, which could be potentially reduced, and the zone of total destruction caused by the initial impact. Furthermore, this method of analysis allows for the testing of different therapeutic strategies against different areas of injury using the technique of quantitative image analysis.

Although the utilization of an injection needle in the present study to deliver putative therapeutic agents was invasive, this method assured the direct delivery of the various factors to the injury site. Based on previous data from brain injury studies (Fisher et al., 1995; Dietrich et al., 1997), intravenous infusions may also be considered as a method of drug delivery in the future. Potential problems including systemic side effects or unpredictable rates of drug delivery to the injury site may limit this method of drug delivery. However, systemic infusion is certainly the easiest in terms of clinical application, and has been used with bFGF in a clinical trial for acute stroke (S.P. Finklestein, personal communication, 1998). Intrathecal infusion remains another possibility, although
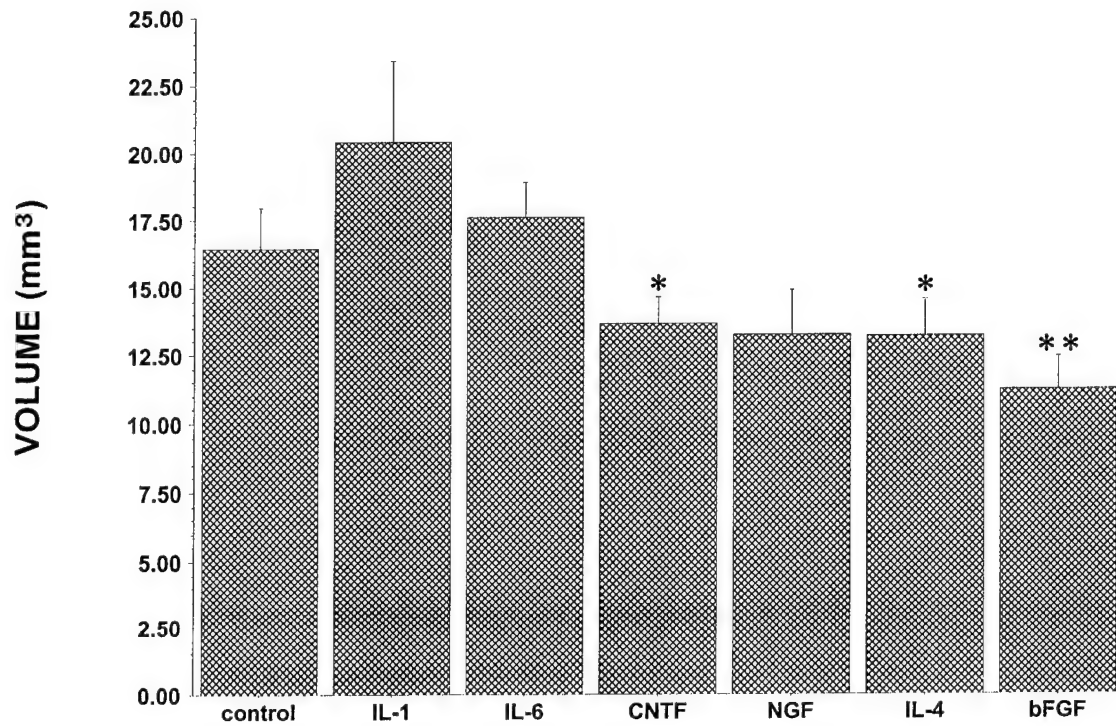
FIG. 3. Effects of drug infusion commencing 1 h postinjury and continuing for 7 days on the mean volume for the zone of partial preservation (ZPP). Compared to infusion of vehicle (control), bFGF significantly reduced the ZPP. Although the effects of CNTF, NGF, and IL-4 were not significant, they did produce a reduction in the volume of ZPP. $^{**}p < 0.01$.
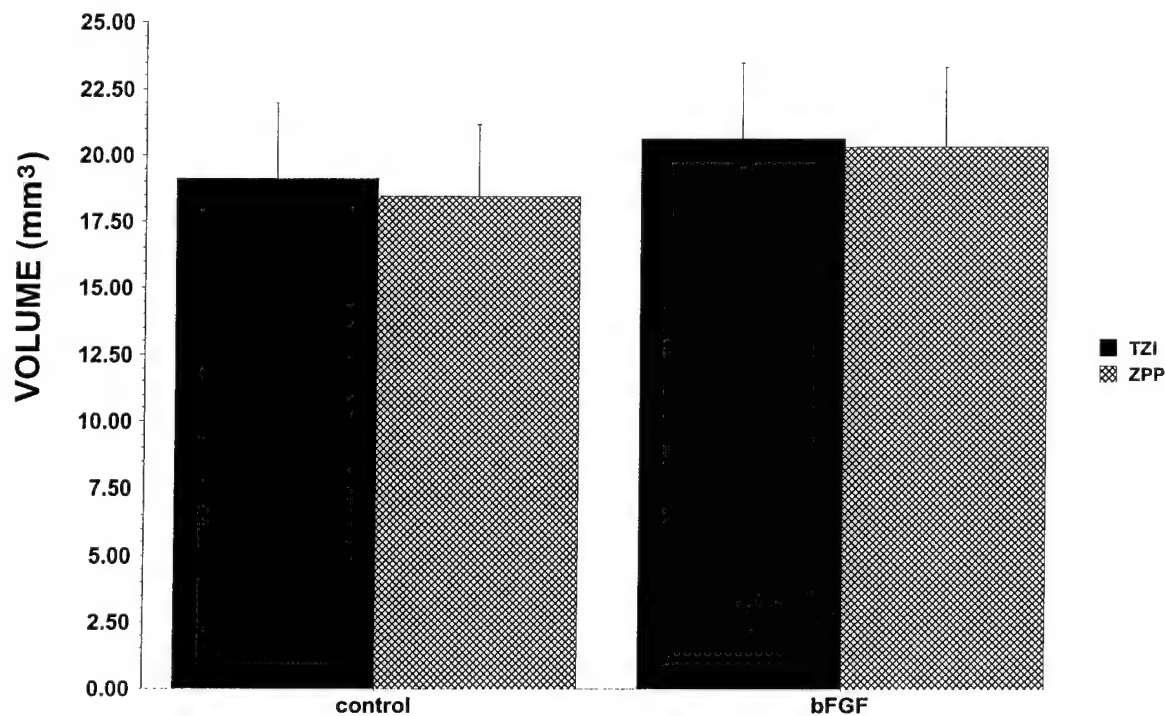

FIG. 4. Effects of bFGF infusion commencing 3 h postinjury and continuing for 7 days. No significant difference was observed between animals infused with vehicle and those receiving bFGF on either the total zone of injury (TZI) or zone of partial preservation (ZPP).

site-directed drug delivery is not achieved with this approach.

Both IL-1 and TNF are primarily the products of monocyte/macrophage/microglia lineage of cells. IL-1 stimulates monocytes and macrophages in an autocrine and paracrine fashion. T and B cell activation is another function of IL-1. IL-1 and its receptors have been widely characterized and mapped in the brain (Yabuuchi et al., 1994), and appear to play a significant role in thermoregulation. IL-1 may also regulate neuron-glial, and glial-glial interactions (Hannum et al., 1991). Significant elevations in IL-1 have been demonstrated after experimental ischemia and trauma investigations and following clinical head injury (Relton et al., 1997). In addition, some benefits were reported after brain injury with administration of the recombinant IL-1 receptor antagonists (Hannum et al., 1990; Relton et al., 1997). IL-1 has been reported to induce intercellular adhesion molecule–1 (ICAM-1) expression and neutrophil-mediated immune responses (Birdsall, 1991). Although there was a trend toward increased lesion volume in IL-1–treated rats versus vehicle, there was no statistically significant difference between groups. The administration of neutral antibodies directed against IL-1 or corresponding receptors may still prove beneficial in the treatment of acute SCI.

IL-3 and IL-4 are T-lymphocyte derivatives and primarily activate B cells (Lee et al., 1993). Both cytokines have been reported to stimulate peripheral monocyte and microglia growth, and activate ICAM-1 and lymphocyte function associated antigen (LFA-1) on microglial surfaces (Lee et al., 1993b). Possible surface mitogen-mediated cellular and humoral immunity may ensue. IL-4 has been observed to downregulate monocytes and neutrophils (Lugering et al., 1997; Niiro et al., 1997). Their direct effects on neurons have not been fully characterized. In the present study, IL-4 reduced TZI by 22% and ZPP by 20%, though the reduction was not statistically significant possibly due to the small sample size. Considering the neuroprotective effects of the potent anti-inflammatory agent IL-10 (J.R. Bethea et al., unpublished observations, 1998; K.L. Brewer et al., unpublished observations, 1998), the anti-inflammatory effects of IL-4 may have accounted for some of the reduction of injury volumes in the present study (Figs. 2 and 3).

IL-6 is produced by monocytes, macrophages, fibroblasts, activated T and B cells, and astrocytes *in vitro* (Lee et al., 1993b). The target cells include pleuri-potential progenitor, B cells, and cytotoxic T cells. IL-6 has been reported to sustain both astrocyte and neuron survival *in vitro* (Kushima and Hatanaka, 1992; Maeda et
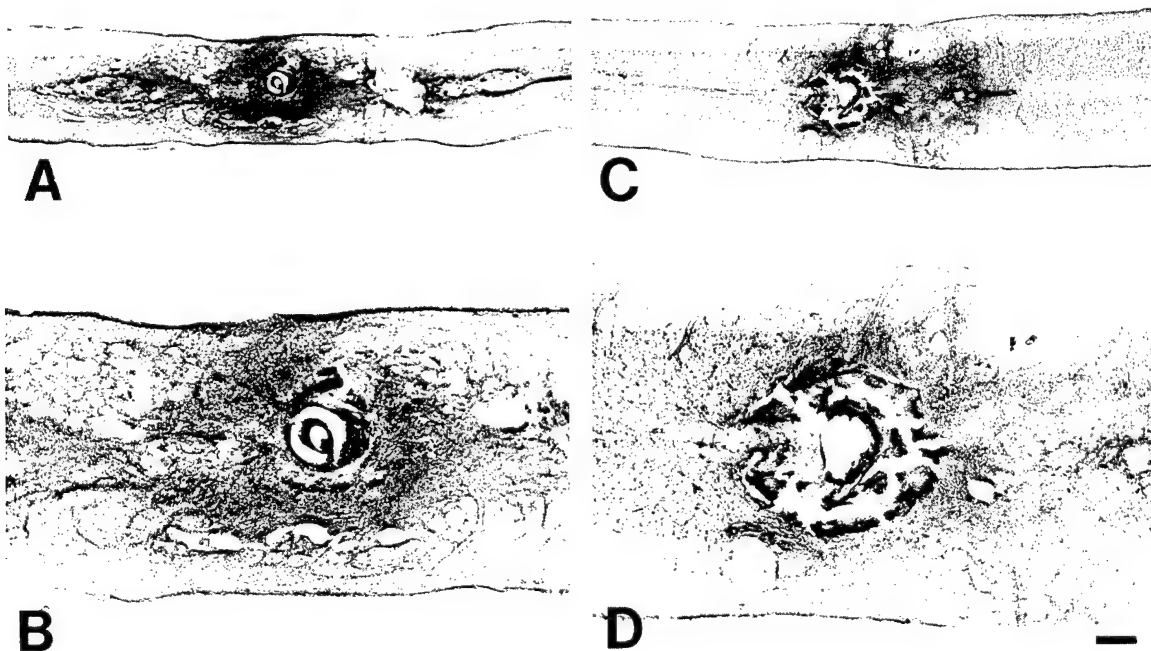
FIG. 5. Sections taken through the epicenter of the weight drop injury after infusion of vehicle (A,B) or bFGF (C,D). Note the large area of damaged gray and white matter in the vehicle-treated cord. By contrast, the area of gray matter damage in the bFGF cord is smaller and there is increased preservation of white matter. The area of total injury (white and gray matter) is outlined with arrows in (A) and (C). Bar = 915 μm (A,C) and 355 μm (B,D).

al., 1994) and appears to interact with a subunit of the CNTF receptor (Saad, 1991). Generally considered to be pro-inflammatory, IL-6 has been reported to promote the survival of acetylcholinesterase (AChE) positive neurons in embryonic rat spinal cord cultures (Kushima and Hatanaka, 1992) and enhance neuronal survival from hypoxia/reoxygenation injury (Maeda et al., 1994). Increased cerebrospinal cord levels of IL-6 have been observed in both adult and pediatric head injured patients (Bell et al., 1997; Relton et al., 1997). Ras-GTP complex accumulation in pheochromocytoma cell lines was induced by IL-6 as well (Nakafuku et al., 1992). IL-6 was shown in the present study not to be neuroprotective after contusion injury.

Both neurotrophins NGF (Blottner and Baumbarten, 1994; Holtzman et al., 1996; Oudega and Hagg, 1996; Saad et al., 1991; Sariola et al., 1994) and CNTF (Blottner and Baumbarten, 1994; Hefti, 1997) trigger neuronal regeneration and induce neuronal differentiation. NGF protects against ischemic brain injury *in vitro* (Holtzman et al., 1996). Its protective action for cholinergic neurons has also been demonstrated (Quirion et al., 1991). The $CNTF_a$ receptor is homologous to the IL-6 receptor (Sariola et al., 1994) in that similar dimerization mechanism of their receptors have been reported. Both have been utilized for the experimental treatment of neurodegenerative disease with variable results (Blottner and Baumbarten, 1994; Hefti, 1997). Trends toward reduction in TZI and ZPP were observed for CNTF- and NGF-treated versus vehicle. The difference was not statistically significant possibly due to the small sample size.

FGF stimulates neuronal proliferation and sustains survival (Hefti, 1997; Murphy et al., 1994; Olson, 1994), but apparently inhibits differentiation (Murphy et al., 1994). FGF also activates rat pheochromocytoma PC12 cells (Nakafuku et al., 1992), and has been reported to enhance the growth of fetal cerebral cortex, hippocampus, and spinal cord neurons (Olson et al., 1994). FGF is a powerful stimulator of angiogenesis, and promotes wound healing. Recently, Cheng et al. (1996) successfully utilized acidic FGF (aFGF, FGF-1) as part of the growth medium for nerve grafts after rat thoracic spinal cord transection. Basic FGF or FGF-2 has been reported to be neuroprotective following experimental focal ischemia and traumatic brain injury (Fisher et al., 1995; Dietrich et al., 1996; Ay and Finklestein, 1998). In recent studies, bFGF together with methylprednisolone treatment was also found to improve behavioral recovery after spinal cord compression (Baffour et al., 1995) and both bFGF and aFGF protect cholinergic neurons following contusion SCI (Teng et al., 1997). bFGF stimulates astrocyte proliferation, and may mediate glial and neuronal interactions important to cell survival (Giulian,

1988). In the present study, bFGF was found to significantly reduce both the total zone of injury and the zone of partial preservation (Fig. 5). Based on these and previous findings, we conclude that bFGF is an important candidate for future investigations directed towards establishing a therapy for acute SCI.

Future studies that should be considered in developing a therapeutic strategy for SCI include combining various factors such as bFGF, IL-4, and CNTF with other treatment modalities. For example, Balfour et al. (1995) have reported a synergistic effect of bFGF and methylprednisolone on neurological function after experimental SCI, and the recent success of moderate hypothermia in the clinical treatment of traumatic brain injury (Marion et al., 1997) and in experimental studies (Martinez-Arizala and Green, 1992; Dietrich et al., 1994; Jiminez et al., 1997) suggests the combined use of mild hypothermia with infusion of bFGF. In the present study, bFGF delivered 3 h after injury did not exhibit neuroprotection, but further experiments to better delineate the therapeutic window for bFGF treatment need to be performed. Finally, preliminary experiments with intravenous bFGF infusion following contusion SCI have shown favorable results (W.D. Dietrich et al., unpublished observations, 1998), and this route of administration needs further investigation in studies with long-term survivals and behavioral testing to fully evaluate the benefits of this treatment paradigm. Based on the results of the present study combined with other clinical and preclinical data, it is proposed that the use of nerve growth factors, together with anti-inflammatory cytokines should be considered for future application in the treatment of acute SCI.

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Detrimental Effects of Systemic Hyperthermia On

Locomotor Function and Histopathological Outcome

Following Traumatic Spinal Cord Injury In The Rat

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ABSTRACT

OBJECTIVE: Posttraumatic hyperthermia has been shown to worsen neurological outcome in models of brain injury. The purpose of the present study was to examine the effects of systemic hyperthermia on locomotor and morphological outcome measures following traumatic spinal cord injury (SCI) in the rat.

METHODS: Spinal cord contusions (NYU impactor, 12.5 mm, T-10) were produced in adult rats divided into three groups. Group 1 (n=9) underwent whole body hyperthermia (rectal temperature: 39.5^0C) 30 minutes post-injury for 4 hours. Group 2 (n=8) underwent normothermia (rectal temperature: 37^0C) 30 minutes post injury for 4 hours; and Group 3 underwent traumatic SCI with no post-injury thermal treatment (n=10). Twice weekly assessments of locomotor function were made over a 6-week survival period using the Basso-Beattie-Breshnahan (BBB) locomotor rating scale. Forty-four days after injury, animals were perfused and their spinal cords serially sectioned. Sections were stained with hematoxilin, eosin, and luxol fast blue for histopathological analysis. The percentage of tissue damage was quantitatively determined using computer aided image analysis.

RESULTS: The results showed that four hours of post-injury hyperthermia significantly worsened locomotor outcome (final BBB scores: 9.7±0.3, Group 1 vs 10.8±0.4, Group 2, vs. 11.3±0.3, Group 3) and led to an increase in the percentage of tissue damage (32.9±3.2% (Group 1) versus 22.3±2.8% (Group 3).

CONCLUSION: These data suggest that complications of SCI, e.g. fever, infection, leading to an elevation of systemic temperature may add to the severity of secondary injury associated with traumatic spinal cord injury and significantly affect neurological outcome.

Key Words: Secondary injury, Hyperthermia, Locomotor function, Neuroprotection

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Postischemic and posttraumatic hyperthermia has been shown to worsen outcome in both experimental models (5, 8, 13, 16-18, 22, 41, 43) and in patients with brain injury (4, 22, 25, 28, 44, 49). These observations have led to concerns over the histopathological and functional impact of modest to severe hyperthermia in patients with spinal injury. Clinical retrospective analysis has demonstrated that fever is a common complication in patients with spinal cord injury (SCI) and many of these patients develop more than one infection (e.g. respiratory, urinary tract) accounting for systemic elevations in temperature. On the other hand, some patients experience elevated core temperature secondary to injury-induced thermoregulatory problems, deep venous thrombosis, or fever of unknown etiology (52, 7, 42).

Whether fever or hyperthermia is directly associated with worsening of neurological outcome following traumatic SCI has yet to be determined or systematically studied in either the experimental or clinical setting. It is known that ischemic and traumatic brain and spinal cord injury are sensitive to temperature and that small changes in temperature can critically influence morphology and functional outcome measures (8, 17, 18, 19, 14, 60). For example, mild to moderate hypothermia (under 34^0C) alleviates (9, 14, 34, 35), but hyperthermia (over 39^0C) exacerbates (16-18, 41, 13, 43, 5, 31, 8) ischemia- and trauma-induced brain injury. Hypothermia also has a beneficial effect on injury-induced breakdowns of the blood-brain-barrier, release of glutamate, formation of free radicals, release of cytokines, and the inflammatory response to injury (for review see ref. 20). Recent studies have also shown that modest systemic hypothermia significantly improves locomotor function and reduces tissue damage following traumatic or ischemic SCI (60, 36). The purpose of the present study was to examine the effects of systemic hyperthermia on locomotor and morphological outcome measures in a clinically relevant model of traumatic SCI in rats.

4

MATERIALS AND METHODS

Experimental procedures were approved and carried out in accordance with the Guidelines of the Animal Care and Use Committee of the University of Miami.

Surgical Preparation

Twenty-seven female Sprague-Dawley rats weighing 225-275g were used in this study. Anesthesia was induced initially with 4% halothane, 70% nitrous oxide, and a balance of oxygen and continued with halothane (0.5% for trauma, and maintenance). The antibiotic Crystiben (0.01ml/100gms) was administered intramuscularly to prevent iatragenic infection. A flexible thermistor was inserted in the rectum to monitor systemic temperature. Pre-SCI, all animals were maintained at a systemic temperature of 37°C (rectal) using a feedback controlled heating blanket. Post-SCI animals were housed individually in cages containing soft bedding. Animals were treated with Crystiben i.m. every other day for 7 days. Water bottles with extended tubes allowed access to water. Food was placed inside the cage until rats were capable of reaching the standard placement at the top of the cage. Animals were checked daily and bladders were palpated at least twice daily and emptied as required until they regained reflex voiding (60).

Contusion spinal cord injury: Traumatic spinal injury was produced as previously described (60). Briefly, following a T-10 laminectomy animals were positioned in the weight-drop apparatus (NYU Impactor). Two spinal clamps were attached to T8/T9 and T11/T12 spinal processes, respectively. A transducer was placed at the site of the muscle near the spinal column, and the impactor rod (10g) centered above spinal segment T-10. The rod was slowly lowered until it contacted the dura, which was determined by

completion of a circuit that resulted in an audible tone. The cord was then contused with the NYU weight-drop device that released a 10g rod from a height of 12.5mm onto the exposed cord. Impact analysis, including degree of cord compression, velocity, time, and height of weight drop, were recorded by a preset NYU impactor software package (26). After trauma, rats were assigned to one of three groups: Group 1 (hyperthermia), Group 2 (normothermia), and Group 3 (weight drop only).

Hyperthermia and Normothermia Protocol

Systemic temperature was regulated by placing animals in a plexiglass chamber equipped with a heating lamp, humidifier, and oxygen supply. The heating lamp was controlled by a servo-regulated temperature controller to maintain the target systemic temperature (rectum) of each animal. Beginning 30 minutes post-trauma, rats were allowed to awaken and were placed into the plexiglass chamber and a flexible thermistor was inserted in the rectum for temperature monitoring. Animals in each group were kept in the chamber for 4 hours. During and after treatment, rats were administered subcutaneously 10ml of isotonic saline to avoid dehydration. Group 1 animals (n=9) underwent mild hyperthermia (rectal temperature: 39.5^0C) starting 30 minutes post-injury for 4 hours. Group 2 animals (n=8) were maintained at a normal rectal temperature (37^0C) beginning 30 minutes post-injury for 4 hours (normothermic group). A second control group (Group 3) underwent injury alone (weight-drop only) with no efforts to regulate temperature during the post-injury survival period (n=10).

Behavioral assessment:

Open-field locomotor function was evaluated using the Basso-Beattie-Breshnahan (BBB) locomotor rating scale (6), a multiple function test of locomotor function which

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provides an efficient and unambiguous locomotor rating. Briefly, non-injured rats were exposed daily for one week to the behavioral testing environment in order to acclimate to open-field exploration. Each rat was tested for 4 minutes with two examiners observing both fore- and hind-limb movements. Postoperative open-field testing for all animals occurred twice a week from Day 2 post-injury to Day 44 post-injury (60). Both examiners were blinded to the type of treatment received by each animal.

Histopathological assessment:

Forty-four days post-SCI, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with isotonic saline for 5 min, followed by a mixture of 4% formaldehyde, glacial acetic acid and methanol (FAM) 1:1:8 by volume for 30 minutes. Following perfusion, the vertebral column with the cord were immersed in FAM at 4°C for 24h. Spinal cords were then removed and embedded in paraffin. Blocks containing the contusion epicenter as well as non-injured tissue at both ends of each cord were analyzed. Serial cross-sections (10μm) were taken from each block of cords taken from animals in Groups 1 and 3 and stained with hematoxylin, eosin and luxol fast blue for histopathological analysis. Tissue from animals in Group 2 was cut longitudinally and stained as described above. For quantitative analysis of tissue damage a total of 20 sections sampled rostrally and caudally from the injury epicenter were examined (Groups 1 and 3) with light microscopy by investigators blinded to the experimental treatment. Reconstructions of the cross-sectional area of tissue damage was made with the aid of an overhead projector (60). The area of injury was easily identified by the presence of reactive astrocytes, microglial, and macrophages. At the site of injury there was also infiltration of inflammatory cell types (neutrophils, leucocytes) (60). The percentage of damaged tissue area in each section was quantitatively determined using

computer aided image analysis (Meta-Morph Imaging System, Universal Imaging Corporation).

Statistical analysis:

All data are expressed as a mean ± S.E.M. and analyzed using a commercially available computer program (StatView). For each rat, BBB scores from each hind limb were averaged together to yield one score per test session. BBB scores and mean percentage of damaged tissue area were compared between the hyperthermia and control animals at each time point using the Student t-test. Differences were considered statistically significant at $p < 0.05$.

RUSULTS

Injury parameters of the NYU weight-drop device used to produce contusion injury are shown in Table 1. No significant differences in velocity, compression, height and time were found between control (normothermia and injury alone) and hyperthermia-treated groups, indicating similar injuries to all animals. Since there was no significant differences in outcome measures between animals in the normothermia and weight-drop only groups, data from these two groups related to BBB scores and contusion parameters were combined and will be collectively referred to as the "control group".

Behavioral Assessment

Results of the BBB locomotor assessment are shown in Figure 1 and Table 2. Immediately after SCI, all animals showed bilateral hindlimb paralysis, as previously documented using the NYU impactor (6, 60). Both control animals and animals undergoing hyperthermia had little or no hindlimb movements 5 days post-trauma and

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then demonstrated a gradual recovery over the next two weeks. By 3 weeks post-trauma, most control (i.e., normothermia and injury alone) animals were consistently exhibiting stepping behavior. Hyperthermia-treated animals achieved only modest improvements demonstrating plantar placement of paws, consistent weight supported dorsal stepping, or occasional weight supported plantar steps. Twenty-three days after injury, ratings of locomotor performance in animals undergoing hyperthermia were significantly below that of control animals ($p=0.015$). This trend continued throughout the duration of the evaluation period (Fig. 1). On day 44 after injury, the final mean BBB score for hyperthermia treated animals was significantly lower than that of control animals (9.7 ± 0.3 versus 11.1 ± 0.2 for the control group, $p=0.0028$; Table 2).

Histopathological Assessment

The results of the histopathological outcome assessment are shown in Figures 2-3. Hematoxylin, eosin and luxol fast blue-stained sections showed that there was significant tissue loss at 44 days after SCI in both control and hyperthermia groups of animals (Fig. 3A,B). For quantitative analysis the amount of cross-sectional damage in cords from animals in Groups 1 and 3 were determined. A comparison was made between these two data sets. Since tissue from Group 3 animals was cut longitudinally it was not possible to include these data in the comparison. As there was no statistical difference in BBB scores between animals in the normothermia (Group 2) and weight-drop only (Group 3) groups it was assumed that there was a comparable degree of tissue damage in these two groups. Following hyperthermia the percentage of damaged tissue was significantly higher than that in control rats at 44 days post-injury ($p=0.025$, Fig. 2). There was a 47.5% difference in contusion volume between the hyperthermic and weight-drop only animals.

Representative examples of sections taken from animals in the weight-drop only versus hyperthermic groups are shown in Figure 3A and 3B respectively.

DISCUSSION

In the present study, using a standardized and clinically relevant model of spinal cord injury (6, 40, 60), the elevation of systemic temperature to 39.5^0C 30 minutes post-trauma for 4 hours resulted in a difference (compared to control animals) in locomotor outcome scores from 2 weeks to 6 weeks after injury. Additionally, on day 44 after injury systemic hyperthermia produced a 47% increase in the area of spinal cord tissue damage compared to weight-drop only animals. These results demonstrate that systemic hyperthermia significantly worsens locomotor outcome and histopathology following traumatic SCI in rats. To our knowledge, this is the first evidence documenting the detrimental effects of secondary hyperthermia on these outcome measures following traumatic SCI.

The pathological sequelae associated with traumatic SCI includes the primary injury and a progressive secondary injury cascade that begins with the elevation of excitatory amino acids (38). Importantly, the N-methyl-D-aspartate receptor (NMDAR), nitric oxide (NO), free radicals, and inflammatory responses are known to play important roles in the progression of secondary injury initiated by brain and spinal cord injury (21, 55, 1, 29, 23, 30, 32, 27, 11, 12). The pathologic mechanism(s) responsible for deterioratation of tissue during and following hyperthermia are not known. Recent studies, however, have demonstrated that brain temperature is a crucial factor in the development of secondary events and neuronal damage following ischemic and traumatic brain injury. For example, release of glutamate (39, 24), lipid peroxidation (56), activation of NOS (3, 48, 33), formation of free radicals (23, 30, 33) and inflammatory

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responses (11,12, 58, 54) in ischemic and traumatic brain injury are temperature-dependent processes, which are accentuated by hyperthermia (30, 11, 50, 53, 24, 10, 3) and diminished by hypothermia (9, 11, 12, 23, 48). Clinically, Reith et al. (45) in a prospective study reported that each 1°C increase in body temperature increased the relative risk factor for poor outcome by 2.2 in 390 stroke patients.

Mild hypothermia (32^{0}C) alleviates ischemia- and trauma-induced brain damage; an effect exacerbated by hyperthermia (9, 14, 34, 35, 17, 18, 41, 13, 16, 43, 5, 8). Similar observations regarding the beneficial effects of hypothermia on secondary damage following traumatic or ischemic SCI have been made in recent studies (60, 36). These studies indicate that secondary damage following traumatic SCI can be altered by temperature manipulations and are therefore relevant to the potential mechanism responsible for the detrimental effects of hyperthermia.. At this time we can only speculate about the role of hyperthermia in these processes following SCI, and additional studies will be needed to determine the precise effects of hyperthermia on different components of the secondary injury cascade (22).

Recently, a clinical retrospective analysis demonstrated that fever, infection, or both occurred at some time during hospitalization in 45-67% of patients with SCI (7, 52, 42). In 77% of the cases only one cause was identified, and in 8% no cause could be defined. The most common causes of fever were urinary tract infection (44%) and soft tissue infection (11%) with respiratory infections accounting for another 5-20% of elevated systemic temperatures. Some patients have other causes including thermoregulatory dysfunction, deep venous thrombosis, or elevated temperatures of unknown etiology. The results of these studies demonstrate the high incidence of fever in patients with SCI. Recently, Schwartz et al., (49) reported that fever occurred in 91% of patients with intracerebral hemorrhage at least once during the first 72 hours after

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hospitalization. Although not as dramatic the incidence of fever in patients with subarachnoid hemorrhage and closed head injury is also high (45, 2, 47). It is therefore possible that fever may represent a natural consequence of CNS insult (perhaps secondary to systemic inflammation). Additional studies will be needed to determine the precise mechanism of this response. Although there have been no systematic clinical studies related to the neurological effects of fever following SCI, the present experimental data provides the first evidence that mild hyperthermia worsens functional outcome and exacerbates tissue damage following traumatic SCI. Thus, fever may worsen locomotor outcome and histopathology of spinal cord injury, and therefore aggressive steps should be taken to prevent the onset of fever and immediate steps should be taken to combat it during the period immediately after injury.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

FIGURE 1:   Time course of locomotor recovery as measured by Basso-Beattie-Breshnahan (BBB) locomotor scores in hyperthermic and control animals. The mean BBB scores of animals receiving hyperthermia 30 min after trauma for 4 hours are represented by the filled circles and control (normothermia and weight-drop only) animals are represented by triangles. Data are represented as mean $\pm$ SEM. *p<0.05, **p<0.01 and ***p<0.001.

FIGURE 2: Comparison of areas of spinal cord damage following hyperthermic treatment or weight-drop only. Sections were stained with hematoxylin and eosin and luxol fast blue for histopathological analysis. The percentage of damaged tissue area was quantitatively determined using computer aided image analysis. Hyperthermia-treated animals (right bar) received whole body hyperthermia beginning 30 min after trauma for 4 hours. Control animals (left bar) received traumatic injury only. Data are represented as mean ± SEM. Compared with control, *p<0.05.

FIGURE 3: Photomicrographs of histological sections following traumatic spinal cord injury. (A) Section taken from an animal undergoing weight-drop only. (B) Section taken from an animal undergoing systemic hyperthermia starting 30 min after injury for 4 hours. Note increased area of tissue damage (B) in the central core of the section and the increase in number of white matter vaculozations following hyperthermia. The area of injury was easily identified by the presence of reactive astrocytes, microglial, infiltration of inflammatory cell types (neutrophils, leucocytes) and by the presence of macrophages. Scale bar in (A) equals 200μm.

Table 1.   Contusion Parameters (n=26)

	Control	Hyperthermia	p^a
Compression	1.69±0.05	1.74±0.07	NS
Height (mm)	12.3±0.08	12.4±0.11	NS
Velocity (m/sec)	0.49±0.002	0.49±0.003	NS
Time (msec)	46.7±0.88	47.7±1.24	NS

Values are means ± S.E.M.
[a] Student t test
NS: Non-significant

Table 2.   **Mean BBB Scores**

Days Post-Trauma	Hyperthermia (n=9)	Control (n=18)
2	0.00±0.00	0.03±0.03
5	3.06±0.92	2.31±0.60
9	8.79±0.37	6.86±0.64
12	9.60±0.31	8.72±0.56
16	9.66±0.30	10.44±0.26
19	9.79±0.41	10.43±0.27
23	10.19±0.40	11.09±0.14
26	9.96±0.32	10.96±0.16
30	9.99±0.31	11.21±0.17
33	9.8±0.33	11.18±0.22
37	9.84±0.35	11.18±0.21
40	10.02±0.30	11.04±0.27
44	9.69±0.32	11.06±0.24

FIGURE 1

FIGURE 2

FIGURE 3

% Area of Damage

40 35 30 25 20 15 10 5 0

*

■ Weight Drop Only (n=8)
□ Hyperthermia (n=8)